

Michael Willis Chase, Declared Witness Testimony: Disclosure Document

PREFACE

I, **Michael Willis Chase** in my late thirties (of age) had a goal: to join the **United States Navy** to become **8404 Corpsman**, then to join the **Freemasons**, and finally to join the **Central Intelligence Agency (CIA)** to be employee of the **Witness Protection Program**. I asked the **Creator** to be with me on this journey, and the **Creator** still is with I on this journey. When I served (active duty) in the **Navy**, I was surrounded by the **Freemasons**, and was told that "**I will go far**". I was invited to, and brought to **Masonic Temples** in Jacksonville, North Carolina and Morehead City, North Carolina.

[Morehead City, North Carolina has separate **Black** (skin) and **White** (skin) **Temples**, for the record.] When I entered the **Navy Reserves**, I accepted the Masonic first degree "Entered Apprentice" at the Masonic Temple #0405 in Morehead City, North Carolina [NC OFFICIAL FORM 20] (See: Exhibit_Masonic_Contract).

I was accepted by the **Senior Military Freemasons**, and I was pulled into **Top Secret** locations and **paid in cash money**. I handled **Top Secret Documents** under direction of the **Senior Military Freemasons** (I did **not** have any kind of **orders** from the Navy about activities, and I do **not** have the proper **security clearance** from the **Department of Defense** to do such things). I was assigned to a **Government Contractor** on new construction of an underground base on **Marine Corps Base Camp Lejeune**, North Carolina. This is where I did finish concrete work, and I was put on painting crew. Another location I was invited to was the **Marine Corps Air Station Cherry Point**, Havelock, North Carolina **Top Secret Hangers**. When I asked if I can take **photographs**, I was told to take as many **photographs** as I want, so I **photographed** military drones with **Aircraft Registrations (Tail Numbers)**. I did post a **photograph** on **social media**. (See; Exhibit_Drones_Tail_Numbers) I lost my **Military Identification Card**, and had to get a New **Military Identification Card** issued at **Marine Corps Air Station Cherry Point**, Havelock, North Carolina (there is a military record of this). I have been to a **Nuclear Missile Base** several times (See: Exhibit_GlassDomeSerialNumbers), at the **Nuclear Missile Base** I **volunteered** for an **experiment** that went *beyond the third dimension* (classified). I will **not** disclose the location of the **Nuclear Missile Base (National Security Risk)**, but I can submit to a **polygraph** test on everything stated in this document. I eventually found out the **Freemasons** worship the "**Fallen Angle**" (**Devil, Satan, Lucifer, &c.**). I witnessed the **Freemasons** lying to the people (the people are called "**cattle**"). When I announced that I Am, leaving the **Freemasons** (because of the **worshiping** of the **Fallen Angle**), and I was going to **tell the people**. I was **attacked** by Member that signed my **Masonic** contract: **Ron Gore** of Atlantic Beach, North Carolina (Ron served +40 years in **United States Marine Corps**). Witness to Ron's attack on me is a woman named **Tina** (bartender) at **Tackle Box Tavern**, Atlantic Beach, North Carolina. I was suddenly fired from all of my employment (Atlantic Beach Fire Department, Tackle Box Tavern, The

Shark Shack, Atlantic Beach, North Carolina (presumed Freemason involvement). I had then taken *refuge* on a friend's (**Earl Weeks**'s) fishing boat until I was financially able to return to Northern California.

Northern California was good for me, for a while. I ran my own small organic farm, old growth fruit trees, vegetable/fruit garden, turkeys, chickens, ducks, volunteered in the community with a dozen or so non-profits, KZFR radio, and was the Deputy Director for NORML for three (3) counties (Butte, Glenn, Tehama) of Northern California. I had met with **County Of Butte District Attorney Michael L. Ramsey**, and I would speak at City Hall Meetings involving city politics. Videos of speakings at City Meetings are on public Facebook: Michael Willis Chase (I Am currently locked out of account, but it is still public).

2018-11-05 I, Michael Willis Chase worked for the Elections (2008, and 2018), as a Provisional Inspector Officer for Electronic Voting Systems for the County of Butte (Northern California). I was *assigned* to the **Masonic Temple** in Chico, California. I was given a meeting time to set-up for the elections at the Masonic Temple the night before the elections, and I arrived early (one hour before meeting time).

Previously, I was in *contact* with a local civilian **Freemason (Brad Marr – General Manager Toyota Chico, California)** that told me, “*Nothing would be going on a temple, it will be on lockdown, because of the public elections happening*”... **Brad Marr** knows about the segregated Mesonic Temples, as the **Black (skin) Masonic Temple in Live Oak, California**.

When I entered the **Chico Masonic Temple**, to my *surprise*, there was a Masonic meeting happening in the office. I had brought a *letter* with me from the **Masonic Temple #0405** in Morehead City, North Carolina to identify myself, besides the **HANDSHAKE**. (I was a *rouge Freemason* member at this point in time, I had not *renounced* from the **CULT** yet...) I explained, I Am working for the elections, and I Am waiting for employees of the elections to setup voting for “**the cattle**” (the public)... I was told to wait in lobby for election officials. I walked to lobby doors, and sat down (I did not enter the public lobby, I sat by the lobby doors, I was still inside the Temple, and just an ear pitch away from the meeting). I started recording with my cellular phone application. As I recored the meeting, all I could think of was “**God bless America**”... The recording produced, “**Embezzlement, Cooking the Books, unethical accounting practices, and a joking threat**” An Electrician arrived during the recording, one of the member **Freemasons motioned towards me** and said, “**Get a bucket of water to stand in, so we can see his soul**” The joke about *torture techniques*, and I did not find it funny at all (everything recorded). Then, an election official arrived, entered the temple, and **introduced himself with district number**. I was *nervous* since I was recording, I stood up introduced myself with **Masonic Temple number “0405”** (on recording). I went to work, setting up for the elections. (See; Exhibit CD: Masonic_Embezzlement)

I realized, I was under an oath to the American people, because of the elections. I had to do the right thing, report what I had heard, what I recorded. So, I reported to the **Federal Bureau of Investigation** (FBI) Chico, California. I was familiar reporting to the **FBI**, I previously reported **Child Abuse case**, and I reported of **Sex Trafficking** by Maintenance employees at the “**Silver Dollar**” **Butte County Fairgrounds** involving work community service applicants (**Witness**: cousin **Steven Whitmarsh**, President of Bearing Belt & Chain, Marysville, California).

How I Lived: I rented out the main house on the property, and lived in a recreation vehicle on the back property. Through a property management, the front house was rented to single father that had his two (2) children every other week. The tenant (Steve) was a singer in three (3) music bands that performed locally. After being social one night, **I discovered that two (2) of the band members were Freemasons**. One (1) of the **Freemason members** struck up a conversation with I, about how the “**Fallen Angle is a powerful supreme being**”... After few months of *conversing* with the **Freemason members**, I disclosed that I Am a **renounced Freemason** (*publicly renounced Freemason on Social Media on December 7, 2018*). In February, 2019 my recreation vehicle was **burned to the ground**, when I arrived to the property the following morning, I inspected the ruins, and was happy to see my cat. I walked out to the front of the property, to the street, to greet the morning Sun, and to thank the Creator for my life... Then a I heard a voice saying “You fucking freak”, as I turned around, I was attacked by tenant (Steve) with brass knuckles. My teeth, upper jaw crushed by the impact of brass knuckles, fracturing my cheek bone, and fracturing my nasal passage with my blood everywhere. (See; Exhibit_Broken_Jaw) I fell to the ground, to my hands and knees, and thought I was going to be attacked more. I pushed myself up (as I was on my knees), my hands came together, and I said, “**I forgive you**”... I do not know why I said this, the words just came out, I feel the Creator spoke though I, there was no more attack, and **my life was spared**... The neighbors telephoned for help, ambulance and police arrived. I was **handcuffed**, and put in ambulance (*I have submitted several complaints against the Chico Police Department over harassment, and I was not liked by the police department*). I went through *emergency surgery*, I absolutely refused all drugs, and was billed \$117,000. I released myself from hospital via release form, and went back to my property. While I was in hospital, my property was **ransacked**, I filed police report about thefts, and the report was **ignored by Chico Police Department**. I filed a **Citizens Arrest Form** about tenant (Steve) *assaulting* me, and the report was **ignored by Chico Police Department**. I filed homeowner insurance claim about recreation vehicle fire, theft of tools, bicycles, everything, and I was **denied insurance claim**, **USAA dropped my insurance, and closed my bank accounts** (without reason)... I **felt**, I had to get out of the area, so I did a “*quick-sale*” of land property with my thoughts of moving back to the Big Island of Hawaii, but then remembered **Sedona**, Arizona. I met a family (**Kirk and Bridget Nielsen**) at **Mount Shasta** that said Sedona was a lot like Mount Shasta.

2019-06-01 I decided to drive to Sedona, and I was amazed at the beauty. I figured **Sedona will be a good place to heal, and to be at peace.** I rented a studio apartment, opened back account at **Chase Bank (6666 Route 179, Sedona, Arizona)**, started attending at the Sedona Synagogue Three (3) days a week, and rotated denominational churches on every Sunday. **Life is a blessing.**

2019-09-30 I, Michael Willis Chase lost the **Chase Bank** card that was issued to me. I walked from *tenant residence* (79 South Canyon Diablo Road, Unite 1, Sedona, Arizona) to **Chase Bank (6666 Route 179, Sedona, Arizona)** to report lost **Chase Bank** card. I entered the bank, walked up to counter, and was greeted by **Chase Bank** teller **Derick Oster**. I explained to **Derick Oster** that the lost **Chase Bank** card issued to me MUST be cancelled, and to re-issue another **Chase Bank** card is in *order*. I then noticed **Derick Oster** was wearing a **Masonic "G"** tie-pin...

I looked **Derick Oster** in the *eyes* and said, "**I used to be a Freemason, I Am now a publicly renounced Freemason**".

Derick Oster turned on his *heels* and walked from counter to back area of **Chase Bank**, out of my view. I waited at counter for a *minute*... Then I decided to be *on-my-way*, so I departed **Chase Bank (6666 Route 179, Sedona, Arizona)**.

Upon returning to *tenant residence* (79 South Canyon Diablo Road, Unite 1, Sedona, Arizona), I thought it would be good idea to call **Chase Bank** customer service phone number, to make sure **Derick Oster** cancelled the issued **Chase Bank** card, and re-issued another **Chase Bank** card. When I called **Chase Bank** customer service phone number, I soon discovered that **Derick Oster** did NOT cancel the **Chase Bank** card issued to me, the **Chase Bank** card was still *active*, and I was NOT re-issued another **Chase Bank** card..? So, I made a *complaint* to **Chase Bank** customer service phone agent about **Derick Oster's negligence** about the matter of the lost **Chase Bank** card.

Then, I traveled to the **Synagogue (Jewish Community of Sedona and Verde Valley 100 Meadowark Drive, Sedona)**. I informed **Rabbi Alicia Magal** about my run-in with **Freemason Derick Oster**, and I gave **Rabbi Alicia Magal** the **Chase Bank business card** (See: Exhibit _Derick Oster Card) of employee **Derick Oster (Rabbi Alicia Magal knows that I Am a publicly renounced Freemason (See Exhibit Masonic_Contract))**, and also, **Rabbi Alicia Magal** is holding my **Mesonic apron**, as a symbol at the **Synagogue**.(See:_Exhibit_RabbiAliciaMagal_SedonaSynagogue).

2019-10-07 Knowing that I identified my-self as a **publicly renounced Freemason**, I decided to present a **donation of peace** (because of attempts prior on my life) to the **Masonic organization**. So, I drove to the **Central Arizona Masonic Lodge (534 South 12th Street, Cottonwood, Arizona)**, but the **temple** was closed.

I remembered **Derick Oster**, so decided to travel to **Chase Bank (6666 Route 179, Sedona, Arizona)** to deliver the donation of peace to the Masonic member **Derick Oster**. I presented two checks (#133-\$333, #177-\$333), and also, I presented an invitation to the High Holy Days (Yom Kippur) at the **Synagogue (Jewish Community of Sedona and Verde Valley, P.O. Box 13, 100 Meadowark Drive, Sedona Arizona 86339)**. **Derick Oster accepted** the two checks (#133-\$333, #177-\$333), and **Derick Oster accepted** the invitation to the **Synagogue High Holy Days (Yom Kippur)**, and I left **Chase Bank (6666 Route 179, Sedona, Arizona)** to return to **tenant residence** (79 South Canyon Diablo Road, Unite 1, Sedona, Arizona). (See: Exhibit _Bank_Checks133and177)

Approximately a week, or so, passed, and the donation of peace checks (#133-\$333, #177-\$333) had NOT cleared checking account managed by **Chase Bank (6666 Route 179, Sedona, Arizona, USA)**. I called **Chase Bank** customer service phone number, I made a complaint to customer service agent about **donation checks** (#133-\$333, #177-\$333) being mishandled by **Derick Oster**.

(Note: When this call was placed to **Chase Bank** customer service. I asked the first **Chase Bank** customer service agent to stay “on-phone-line” and listen to conversation with second **Chase Bank** customer service agent, that I was to be transferred to).

Ironically, I was discriminated by second **Chase Bank** customer service agent about complaint, and was rudely “hung-up” on by second agent, but first **Chase Bank** customer service agent was still “on-phone-line” and confirmed she heard everything during entire phone discussion.

Note: I also, made a complaint “on-line” via email to **Chase Bank**, confirmation number ecw19030-060.

2019-11-21 I decided to close the **Chase Bank** accounts. I gathered “**family heirlooms**” from **tenant residence** (79 South Canyon Diablo Road, Unite 1, Sedona, Arizona) as Gold/Silver//Emeralds/Coins/Meteorite/1886 Elgin “Private Label” gold pocket watch, and Gold/Silver Chess Pieces, etc... (See: Exhibit _Photo_GoldWatch_Meteorite), and also I grabbed an “**aluminum art sculpture**” in orange bag (that my **mother** constructed twenty-years-ago) . I had thought out the day, to place “**family heirlooms**” in “safety-deposit-box” at **Wells Fargo Bank** (2201 AZ-89A, Sedona) upon opening new accounts, also to take “**aluminum art sculpture**” in orange bag (that my **mother** constructed twenty-years-ago) to **Ed's Welding and Fabrication Shop (Edward Dison, 2160 Shelby Drive #203, Sedona, Arizona)**, where I conduct business on “patentable” working models of my inventions, and then planned to go on a hike in the beautiful **Sedona** red rock area. Before my “journey of the day”

could start, I needed petrol for vehicle, so after fueling vehicle at Chevron (Village of Oak Creek, Sedona), I proceeded to Chase Bank (6666 Route 179, Sedona, Arizona). I entered Chase Bank (6666 Route 179, Sedona, Arizona), and there were *many* people in the teller lines (**Derick Oster** was one of the tellers). There were other people in building **not** in teller lines, I presumed to be Chase Bank employees, so I asked, “Who is in charge of this Chase Bank (6666 Route 179, Sedona, Arizona)?” One random man said he was not in charge, and then a woman said she was in charge of this Chase Bank (6666 Route 179, Sedona, Arizona). I “chirped” the hiking-whistle that was pinned to shoulder of my *Navy Pea-Coat*, and announced, “I Am, closing my accounts today”. The woman in charge of Chase Bank (6666 Route 179, Sedona, Arizona) said “Come over here (pointing with her finger) to my desk, and (she) can get that started”. I looked over to **Derick Oster** (who was *looking* at me), and I asked, “Derick, when you are done with that customer, can you come over (I pointed with my finger) to this woman's desk?” **Derick Oster** replied, “Yes”. I continued to woman’s desk where I was *greeted*, invited to sit, and was asked for the Chase Bank card, *identification*, on which I provided. **Derick Oster** came over to the desk (of woman in charge), and I asked him, “What did you do with the two checks #133, and #177 I gave you?” **Derick Oster** said, “I do not know what you are talking about”... (Note: **Derick Oster** did admit to police he did have knowledge of both checks 133 and 177.) I felt upset (I knew this man mishandled my **donation checks**, and was he lying. (Exhibit_PoliceReportChecks). I felt like a child being bullied in the playground by a bully. I stated, “I Am a Truth Bomb, call the police, or I will...” Then **Derick Oster** looked at me with a smile on his face and said, “You have a bomb”... With my vehicle key **FOB** (car alarm remote) in hand, I replied, “I have electronic shield device from nuclear missile base (See: Exhibit_PhotoGlassDome) in van, and my "FOB" (car alarm remote) has 333 meter range...” (Thus, is a *true* statement, but **not threatening** in any-kind-of-way, but diffidently *confusing* statement though). Then **Derick Oster** set-off the *alarm*, and then **Derick Oster** abandoned the vessel-building Chase Bank (6666 Route 179, Sedona, Arizona)... One of the *customers*, **Steven Moss** (confessed in police report) threatened to “knock my teeth out.” I pointed to my mouth, and showed him the brace on my already *broken* jaw, and I stated, “They already did that...” (See Exhibit: **Broken_Jaw**, Dr. Kirk Westervelt DMD is dentist that removed my jaw-brace in jail, he can testify of speech impairment of brace, and is still my dentist in Sedona.).

Steven Moss *texting* on his phone, then blocked the door of Chase Bank (6666 Route 179, Sedona, Arizona) **not allowing** other customers to exit the vessel-building Chase Bank (6666 Route 179, Sedona, Arizona). I encouraged **Steven Moss** to move out of the way of the door, with a “locker-room-butt-tap”, and I verbally explained he was **blocking** the door, for customers could **not** exit, and **Steven Moss** **complied**. Then, I was *alone* in the Chase Bank (6666 Route 179, Sedona, Arizona), the alarm going-off, and my *tinnitus* *ringing* in my head. I knew, I **must** stay calm to explain to the first

responder on what just *happened* to me. I asked the **Creator** to be with me, but he *already was...* I picked up a soccer-ball out of the “**Toys-For-Tots**” barrel, and kicked it around the bank to *settle* my nerves, but it did not work, and so I waited...

First responder arrived, who was United States Federal Forrest Ranger **O’Neil**,

I knowing Maritime Law (United States Navy), and Physics (Space-Time).

I stated, “**This vessel-building has been abandoned, I have taken this vessel-building Chase Bank (6666 Route 179, Sedona, Arizona) under maritime law (physics: space-time), I Am the new Captain. Please come aboard to talk.**”

O’Neil replied, “**No**” (*he did not want to come in and talk*).

I was *confused*, why did he not want to talk? I waited, Oneil approached me again, and asked “**what is going on?**”

I began to *explain* the situation, what I was being accused of, and how ridiculous it must sound... “**I have a bomb on me, and in my van...**”

Then came a series of questions from **O’Neil**, “**What is your name?**”

I replied, “Michael Willis Chase”

O’Neil: “**Are the owner of the bank?**”

I replied, “What are you talking about?”

O’Neil, “**Your phone controls the bomb?**”

I felt frustrated on his question, “I have EMP device in van?”
[EMP device protects against lightening strikes, power surges, etc...]

O’Neil, “**Do you want me to call anyone for you?**”

I responded, “My cousin Steven Whitmarsh, he is President of Bearing Belt & Chain Company Marysville California, and you can call Bridget Nielsen of Sedona..?”

I did not need to make a call, I felt that I was honestly communicating with O’Neil’s questions to *de-escalate* the situation.

I felt frustrated from the alarm sounding (tinnitus), all the accusations, the questions.

O'Neil had propped bank doors open with boulders (rocks), and I asked him, "Stop propping doors open." I approached the doors with soccer-ball in my hands, and I was **Pepper-Sprayed by O'Neil**. I was in sudden pain, and could not breath. I tossed the soccer-ball, and picked up boulder (rock) **that had been placed by O'Neil by the doors**. Stepping backwards, I could not see, in pain from **Pepper-Spray**, I tossed boulder out of the bank door, but without knowing the door shut (I could not see), the boulder (rock) went through glass of door.

I felt upset, I could not see, I could not breath, I felt pain from the **pepper-spray**, the bank alarm ringing in my head (tinnitus), and I felt in fear for my life...

I asked O'Neil, "Why did you do that?" (Pepper-spray), and I could **not** hear his response (bank alarm sounding, and tinnitus).

I felt confused, so I just stood in the bank, blinded in pain (pepper-spray), trouble breathing, my head ringing (tinnitus) because of the alarm sounding.

After a few moments, there were voices around me (I could not understand what was being said), I motioning hand and shoulder signal (I do not understand), there was a loud "**Bang**" (my ears ringing) I was shot in the back by an **electric taser-gun**. I felt the electricity in my body, I turned around, and collapsed on the floor.

I felt scared, I stated, "I Am not resisting" while Officers (**agents O'Neil, Hawkins, Smith, Mickle, and Rumpf**) emptied my pockets (Family Heirlooms, Gold Watch, Meteorite, Emerald Ring, DD-214 Honorable Discharge Navy, Passport, etc...), and again, **electric tased me second time** (for no reason), and handcuffed me.

I was booked into County of Yavapai jail as "**Tattooed Middle-Eastern**" ???
I Am NOT Middle-Eastern, nor hispanic, and I do **NOT** have **any tattoos**.
This is called "**RACIAL PROFILING**"

Officers (agents) forced palm/finger prints out of me, and (eye) cornea scan from me by force, against my will, seizing my property without consent.
I refused to signature any documents put in front of me.
(See Exhibit: Tattooed-Middle-Eastern_Booking_Refused_Consent).

Judge Lundy ordered **\$500,000** (half a million dollars) **bail, cash only**.

Violation of excessive bail.

"Bail is 'Excessive' in violation of the Eighth Amendment when it is set at a figure higher than an amount reasonably calculated to ensure the asserted governmental interest."

The accused, Michael Willis Chase stated in the Grand Jury Bond Hearing,

"It is perspective, that you [William N. Lundy Jr] are defending the bank [Fund]."

I was put in the psychotic ward T-7 cell **naked, no water**, and a **hole in the floor** to use as toilet for several weeks. I had to **beg** for water. I had a counselor Mike show up to my cell door (**drunk**) on Thanksgiving (2019). I made complaint about (**drunk**) counselor Mike as interoperate.

After several weeks in psychotic ward, a Lieutenant Sheriff discovered I was writing letters to an agent of the **Federal Bureau of Investigation** (FBI) about my innocence and wrongful imprisonment. I was moved to *maximum* confinement cell (with hard-criminals, murderers, rapists, and inmates of violent crimes).

I have filed one-hundred twenty-two (122) grievances on violations happening inside County of Yavapai jail (I have all the records from jail). **I also filed twenty-one (21) federal civil right complaints by prisoner (form 550/555) to the United States District Court about crimes and violations while I was falsely imprisoned for ten months** (I have all the federal documents filed).

I was able to speak with COUNTY OF YAVAPAI jail “**Chaplin Randy**”, whom I confessed that I Am, a **Renounced Freemason**.

Chaplin Randy Said, “**The Freemasons Worship the Fallen Angle (Satan, Devil, Lucifer, etc...), the Freemasons tried to recruit me, and disclosed Satanic Worship.**”

Chaplin Randy’s statement was heard by the next cell to mine.

Witnesses: **Ryan Welch and Cody James Wilkins**.

My rights were violated as I was **denied** basic needs, **denied** drinking water, **denied** access to legal library, **denied** access to worship, **denied** food (vegan diet), **denied** toilet paper, **denied** outside (fresh air for lungs) **I was outside approximately six (6) times in ten (10) months incarcerated.** **Denied** ink pen to write the courts, and **denied** ink pen to have documents notarized by notary. **Denied** visits with attorney tree times, **denied** due process, **denied** equal protection, and I witnessed (with other witnesses) obstruction of justice (and more for the record).

Judge Michael R. Bluff: “Conflict Of Interest” His Family member is an employee of the international bank [Fund] (JP Morgan Chase Bank) where **The Accused’s property was seized**. Michael Bluff **denied** motions Reduction Of Bail And Release Conditions, and Michael Bluff **admitted** (on the record) his family member is an employee at the international bank [Fund] (JP Morgan Chase Bank) “**Conflict Of Interest**” and he **recused** himself from the case (accused was detained ten months incarcerated under bias Mr. Bluff).

Attempted Murder. Forced drugged (Haldol - Major Tranquilizer) by Judge **Kottke** order with **no evidence** presented at hearing, **that caused abnormal heart rate, and put at my life risk**. Jail **Doctor Mark Jerome Collins** committed perjury under oath by lying about records, that did not exist at time of hearing (as a matter of record, I have

all the jail medical records from the jail that prove **no records existed at time of hearing**).

My costs of over two years have exceeded \$100,000 hiring five attorneys (two appointed public defenders) that did nothing (but tell me to plea insanity), and under threat, duress, and coercion to agree to plea deal. All attorneys are advocates of the state, part of the state bar, oath of loyalty to the court, not “we the people”.

Judge John D. Napper in open court, denied request for *corpus delicti*, **denied** request for verified complaint, **denied** counsel of choice, **denied** psychologist evaluation of prosecuting Attorney (Equal Protection, Due Process, because I was evaluated for competency twice), **denied** answers for Administration and Procedural Matters Act (Obstruction of Justice), **denied** my challenge of jurisdiction of the court, **denied** qualifications of Prosecuting Attorney, **denied** qualifications of judge. Mr. **Napper** Ordered date of October 25th, 2021 deadline for The Accused, Michael Willis Chase to file All motions, briefs, declared witness testimony, and **ALL MOTIONS, BRIEFS, DECLARED WITNESS TESTIMONY** have been **DENIED** as of November 29, 2021.

Cruel and Unusual Punishment, Conflict Of Interest, Violation Of Eighth Amendment, Violation of Due Process, Violation of Equal Protection, Obstruction of Justice, Trespass On The Case, and More for The Record.

I have been studying law full time over the past six (6) months (federal litigation trial law: Title 42, 1983, The Common Law, Civil Rights, Constitution (bondage) Law, Roman (Admiralty Corporate) Law, All the Declarations to be free of King George, **The fourteen presidents before (King George's) General George Washington**, and have discovered America has been dissolved as a matter of law (Executive orders, treaties, the takeover by corporations, World Bank/IMF, &c.). I learned about contracts, and adhesion contracts that I have now rescinded. No Contact (Charter), No Jurisdiction...

I AM, CLEAR ON MY STATUS:

The **Creator's Inalienable (Alien) Inhabitant Rights** that are recognized by **The Law Of Nations**. I Am, I stand with the **Creator**, and **The Law (of Moses)**, *alias dictus The Scripture, The Creator's Law is above ALL of man's laws*. [alias dictus, latin meaning: Otherwise called]

SABOTAGE IN CASE:

[Eighth Commandment: You shall not bear false witness against your neighbor.]

Derick Oster, Marissa Luman Lying to Detectives, *Defamation of Character*. **Both Derick Oster (Freemason), Marissa Luman** (Daughter, and Wife of Freemason) made **un-true comments** in Police Report as...

- Michael Willis Chase was attending Synagogue, raising hell, saying he was Messiah.
- Michael Willis Chase is paranoid schizophrenic who has been off his medications.
- Michael Willis Chase used to date Marissa Luman.
- Michael Willis Chase threatened Marissa Luman's father.
- Michael Willis Chase said Marissa Luman is a devil worshiper.
- Michael Willis Chase causing big scene at Synagogue, asked to stop, kicked him out.
- Michael Willis Chase did not like Freemasons.

THE TRUTH OF THE MATTER IS:

- I, Michael Willis Chase **NEVER** disturbed Synagogue, **NEVER** claimed to be Messiah at Synagogue.
- I, Michael Willis Chase sober minded, do **NOT** take drugs, and do **NOT** drink alcohol.
- I, Michael Willis Chase **NEVER** kissed Marissa Luman, **NEVER** dated Marissa Luman, and Marissa Luman is married to a Freemason.

[Sixth Commandment: You shall not commit adultery.]

- I, Michael Willis Chase has **ALWAYS** been polite to Marissa Luman's father.
- I, Michael Willis Chase **NEVER** called Marissa Luman a devil worshiper.
- I, Michael Willis Chase was **ASSAULTED** by Synagogue member "**Joe**" (**witness June**), and **complaint** emails sent to **Sedona Police Department** (Officer **Wayne Butler** who works for Water District of Sedona now). Also, Sedona Police Officer **Wayne Butler** witnessed abuse from Synagogue woman towards I, Michael Willis Chase on the High Holy Days (Yom Kippur) 5780 (2019).
- I, Michael Willis Chase has Freemason acquaintances as **Samuel Harris** (Served together in United States Navy), on (old public) Facebook account: Michael Willis Chase, and I, Michael Willis Chase does **not** dislike anyone...

Dated this 17th day of December, 2021.




Autograph:

Michael Willis Chase of the Chase Family,
Pro Se, Principal Creditor for
MICHAEL WILLIS CHASE™, which
is a Corporate Identity, a Legal Fiction in
all uppercase, a decedent. All rights reserved.

Seal

Witnessed By: (two or three witnesses)

As: "... at the mouth of two witnesses, or at the mouth of three witnesses, shall the matter be established." Deuteronomy, chapter nineteen, verse fifteen.



Witness

Paul Agneberg



Witness

Carolin Isabelle Hauser

McGraw-Hill
Banking

Derek Oster
Lead Associate Operations

Consumer Banking

AZ1-0474

6666 Hwy 179

Sedona, AZ 86351

Phone:

928 284 1030

Service Line: 800 935 9935







Michael Willis Chase is at **Sedona** ...
Desert.

Oct 7, 2019 • Sedona, Arizona •

Today (in the matrix) ❤️

I Forgave The Freemasons For Attempts On My Life

I Gave Two Forgiveness Checks ? (\$\$\$) ? 🌟

First Check #177(7) 10-07-2019 \$333 North Carolina Premeditated Murder First Degree (angels are close) 😊

Second Check #133(7) 10-07-2019 \$333 California Premeditated Murder First Degree (angels are close) 🙏

Thus, Marking The Freemasons "The Beast"
\$666 👽

Blessings 🔥



Search



Michael Willis Chase is at **Sedona Desert.** ...

Sep 30, 2019 • Sedona, Arizona •

I Was Approached By Free Mason

This Morning

I Filed Official Complaint

#ecw190930-06038

To Protect I Self



#OnLikeDonkeyKong



Hello Michaelwillischase,

Your request (#3116585) has been updated. You can view the update below.

Louise, Sep 18, 9:29 AM PDT:

Hello Michael,

Thank you for contacting the 23andMe Team.
We currently only report on human DNA, and are unable to analyze DNA from any other sources





Michael Willis Chase is at Sedona

...

Desert.

Sep 30, 2019 • Sedona, Arizona • 🌎

I Was Approached By Free Mason

This Morning

I Filed Official Complaint

#ecw190930-06038

To Protect I Self



**CAUTION: NOT TO BE USED FOR
IDENTIFICATION PURPOSES**

**THIS IS AN IMPORTANT RECORD.
SAFEGUARD IT.**

**ANY ALTERATIONS IN SHADED AREAS
RENDER FORM VOID**

CERTIFICATE OF RELEASE OR DISCHARGE FROM ACTIVE DUTY

SPECIAL ADDITIONAL INFORMATION (For use by authorized agencies only)

23. TYPE OF SEPARATION RELACDU AND TRANSFERRED TO NAVAL RESERVE	24. CHARACTER OF SERVICE (Include upgrades) HONORABLE	
25. SEPARATION AUTHORITY MILPERSMAN 1910-102	26. SEPARATION CODE LBK	27. REENTRY CODE P.R.S.

28. NARRATIVE REASON FOR SEPARATION
COMPLETION OF REQUIRED ACTIVE SERVICE

29. DATES OF TIME LOST DURING THIS PERIOD (YYYYMMDD)

30. MEMBER REQUESTS COPY

TL: NONE S. MEMBER REQUESTS COPY
(Initials) *M.W.*



After Visit Summary

Chase, Michael Willis

DOB: 06/29/1971 (47y)

Visit date: April 22, 2019

Date generated: April 22, 2019 13:32

CHICO VA CLINIC

Today's Visit

Clinic Visits

Apr 22, 2019 13:00 - CHICO PACT SIERRA 5 / LEE, JOSEPH TIN-YAM
/ WITHERSPOON, TOM

Providers

- LEE, JOSEPH TIN-YAM
- WITHERSPOON, TOM

Reason For Visit

- Mental health annual physical examination done.

You Were
Diagnosed With

- Mental health annual physical examination done
- Jaw pain
- Tinnitus, Bilateral

Vitals as of This
Visit

April 22, 2019

- Blood Pressure: 110/80
- Pulse: 61
- Pulse Oximetry: 98
- Temperature: 98.9 F
- Height: 71 in
- Weight: 186 lb
- Body Mass Index: 26.00
- Pain: 0

My Treatment Plan

New Orders
From
This Visit

None

Other
Instructions

None

My Ongoing Care

Primary Care
ProviderDorjee, K
"PENDING" CHICO CASCADE 2Upcoming
Appointments

No appointments scheduled in the next 3 months

Immunizations

None

Allergies and
Adverse Drug
Reactions
(Signs /
Symptoms)

No known allergies

My Medications

None

Chico Hearing Aid Center

1600 Mangrove Ave Suite 160 Chico, CA 95926 530-347-8132

noah4

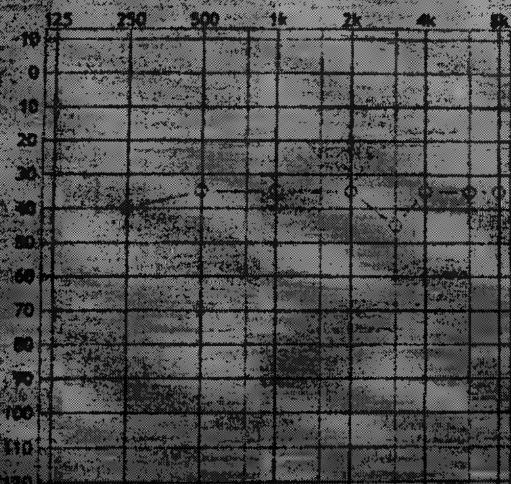


Patient Name: CHASE, MICHAEL
Address: UNK
City: CHICO
Zip code: 95926

6/29/1971

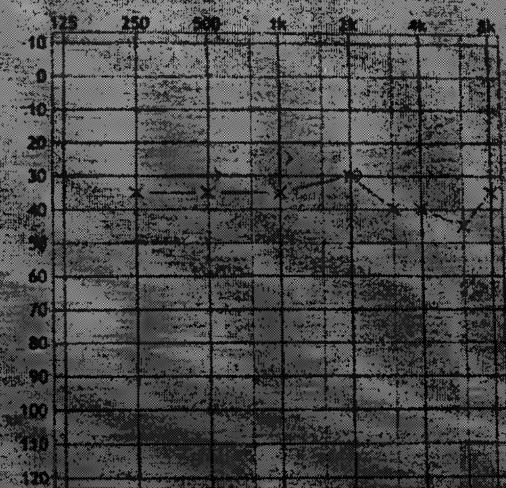
Test date:

4/8/2019



Legend

RBL
AC
BC



Pure Tone Average

AC: 35
Right (O Freq.): 34
Left (X Freq.): 34

4/8/2019

Chase Michael 3884



Common Law Court
Great Britain & International

Book of Deeds

Extract

Birth Certificate

07 October 2021





Common Law Court
Great Britain & International

Birth Certificate

Birth Name

michael willis chase

E-Mail Address

aloha777sedona@gmail.com

Sex

male

Place of Birth

red bluff, county of tehama, state of california,
united states of america

Date of Birth

june 29, 1971

Time of Birth

12:40 am

Fathers Name

arthur willis chase III

Fathers Date of Birth

january 12, 1944

Fathers Address When Child Was Born

247 treasure drive, red bluff, county of tehama,
state of california, united states of america 96080

Mothers Name

carole lynn lopez chase

Mothers Date of Birth

may 11, 1945

Mothers Address When Child Was Born

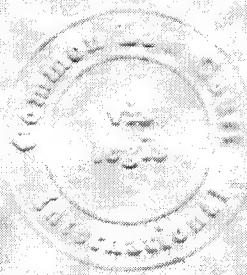
247 treasure drive, red bluff, county of tehama,
state of california, united states of america 96080

Name of Individual Submitting Information

michael willis chase

This Birth Certificate has been recorded for preservation, with the Common Law Court. The information contained within it is based on first-hand knowledge and has been provided by the named individual. This individual has submitted this information under penalty of perjury and full liability.

Book of Deeds, Extract: Birth Certificate, Recorded 07 October 2021





Common Law Court
Great Britain & International

Book of Deeds

Extract

Fictitious Names

13 October 2021

Signature: michael willis chase





Common Law Court

Great Britain & International

Application for Registration of Fictitious Name

1. Fictitious Name Registered

michael willis chase

p.o. box 4461

sedona arizona, usa 86340

2. Region of Principal Place of Business

sedona arizona, usa

3. Individual Owner(s) of Fictitious Name

michael willis chase

p.o. box 4461

sedona arizona, usa 86340

I the undersigned, being an owner of the fictitious name, certify that the information indicated on this form is true and accurate. I further certify that the fictitious name to be registered has been recorded with the Common Law Court for preservation in the 'Book of Deeds'.

I understand that by submitting this application online, I have agreed to the electronic signature below, this application shall have the same legal effect as if made under oath. I am also aware that by submitting false information on this application I would be guilty of committing a fraudulent act, for which I can be prosecuted.

Signature: michael willis chase

Book of Deeds, Extract: Fictitious Names, Recorded 13 October 2021



Common Law Court

Great Britain & International

New

Renewal

Amended

Dissolved

Business Registration and Ownership Certificate For Fictitious Name

The undersigned hereby confirm that the following person (or persons) now owns, conducts or transacts, or intends to own, conduct, or transact a business, or place of business in the region of the United Kingdom, under the name, designation or style set forth below:

1. Name of Business

michael willis chase

2. Address of Business

p.o. box 4461 sedona arizona, usa 86340

3. NAME OF PERSON(S) owning, conducting and/or transacting the above business, and the home address of each:

michael willis chase p.o. box 4461 sedona arizona, usa 86340

4. PARTNER CERTIFICATE. The undersigned hereby certifies, that:

- The business mentioned herein IS NOT a partnership.
- Length of time partnership is to continue (insert either the term agreed by the Partners, or the Statement "Not limited by partnership contract") NA

5. ELECTRONIC SIGNATURES OF ALL PERSONS LISTED ABOVE

Signature: michael willis chase

The individual submitting the APPLICATION FOR REGISTRATION OF THE FICTITIOUS NAME will take full responsibility for the information submitted and will be guilty of FRAUD if providing any false information.
The Common Law Court does hereby certify that the foregoing is a true and exact copy of the original document, recorded in the Book of Deeds.

699-9688

Michael Willis Chase (925) 555-5555

*Living
"Moving To Sedona AZ"*

Sold

Property, 806 Sequoyah Ave Chico CA 95926

*aloha77sedona@live.com
michaelwillis.chase@gmail.com*

I would like to be part of a team in a friendly environment that strives to problem solve, multitask, give excellent customer service, and meet deadlines with a smile. I am dependable, reliable, and have excellent skills.

Medical: Patient Care, Medical Records, Patient Assessment, Vital Signs, Strict Adherence to Patient Privacy, OSHA Trained.

Clerical: Excellent Computer Skills, Microsoft Office, Integrated Accounting, QuickBooks, Quicken, PeachTree software, Experienced in Data Entry. **MAC & PC. Excellent Customer Service**, sales, inventory, work safety, and fundraising. **Excellent verbal and written communication skills**, team player. Preoccupations: Licensed water ski instructor, certified SCUBA & current BLS (CPR & AED), Surf, Skateboard Instructor CalSkateChico, Snowboard, Wakeboard. Current Forklift License

Volunteer 19 Nonprofits - Salvation Army, Chico Library, Peace & Justice Center, NORML, Food Not Bombs, Free Trees, ReStore, Peace Panel Project.

The Salvation Army Church - Security Driver/Money Transport Christmas Season 2015, Handled/Counted over 100K/Cash - Lt. Craig

BBB Hulling Inc. 11675 Dairy Road, Chico, CA 95973 Boss: Gary Barnes, Walnut Hulling and Shelling Services (530) 342-0349

The Tackle Box Tavern 107 Atlantic Blvd, Atlantic Beach, NC 28512 (252) 222-3474- Bartender/barback/doorman Jun '12-Aug'12

The Shark Shack 100 S Durham Ave, Atlantic Beach, NC 28512 - Server/Customer Service/Open/Close restaurant. May '12-Aug '12

Town of Atlantic Beach Fire Department - Ocean Lifeguard, 125 W Fort Macon Rd. Atlantic Beach, NC 28512 (252) 342-7244 May '10 - Sept '10

Friendly Caregivers – Patient Home Care (252) 240-1234 Morehead City, NC 28557 April '10 – May '10.

Hospital Corpsman USN – Camp Lejeune, NC. Contact: HM2 Kisha Kolb USN (910) 581-0042. OCT '09 – Jan '10 (Naval Reserves, Jan '10-Nov '12)

Officer Provisional Inspector Electronic Voting Systems County Clerk-Recorder/Registrar of Voters County of Butte. Boss: Mary 530-538-7761 Nov '08, Nov '18

In-Motion-Fitness. Sanitation, and detail. 5 Diamond service. 530-343-5678. Sept '08 – Nov '08

Butte Creek Brewery Co. Warehouse technician, sanitation, quality control, & efficiency of bottling line. Supervisor, Cooper 530-514-2633. Sept '02 – June '07.

Boreal Ridge Ski Resort Customer Service, sales, inventory, shuttle driver, & snow removal. Boss: Bob Blohm. 530-426-3666 Nov '93 – May '95 & Oct '06 – Jan '07.

Big K-Mart. Shipping & receiving clerk, warehouse technician, inventory, and data entry. Boss: Debbie Bruce. 530-345-9461. Aug '00 - April '01.

U.P.S. Data entry, tracking inventory, quality control, customer service, strict adherence to safety standards, employee of the month. Supervisor, Max Parsons. 530-378-2536. Sept '94 - June '98.

EDUCATION:

United States Marine Corps Camp Lejeune, NC. Field Medical Service Technician, Class 10020 - 05 Mar 2010.

Naval Hospital Corps School Great Lakes, IL. Hospital Corpsman Basic - 08 Oct 2009

Accounting Applications Certificate. Northstate Business College Inc. Chico, CA

SEARCHED AND INDEXED

AL ALAM

SEARCHED NO. 42



SEARCHED AND INDEXED
SEARCHED AND INDEXED
SEARCHED AND INDEXED
SEARCHED AND INDEXED

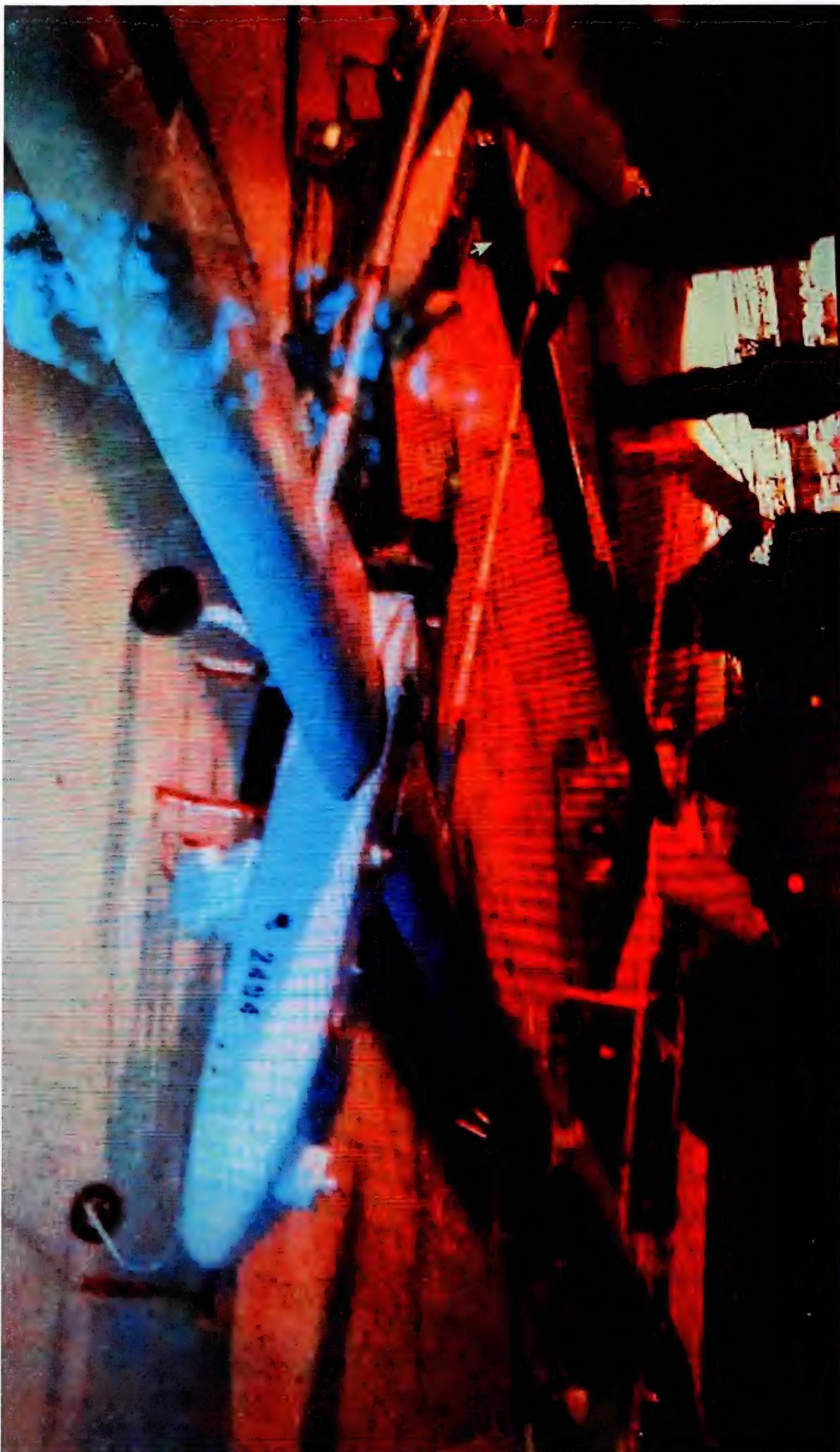
Happy
Birthday



RECORDED & INDEXED
O. BOX 389
C. 20557

SEARCHED

INDEXED





Ocean Lodge No. 405
A.F. & A.M.
MOREHEAD CITY, NORTH CAROLINA

G

NOTICE OF ELECTION TO RECEIVE THE DEGREES

Ocean

A. F. & A. M.
Morehead City

September 14

Lodge No. 405

North Carolina

20 10

Mr. Michael Willis Chase
104 B Kinston Ave
Atlantic Beach, NC 28512

Dear Sir:

I take pleasure in notifying you that on September 14, 2010 you were elected to receive the degrees in Masonry. You are requested to present yourself at the lodge room located in Morehead City, N.C. on September 21 at 6:00 P.M. for the degree of Entered Apprentice

If for any reason you cannot appear as at the time stated herein, please notify me soon as possible.

Your truly

J. Hall

Secretary

NOTES: 1. This form may be altered or changed to suit the need of a particular case
this notice should be kept by the secretary for at least one year.

(77-01; 77-02).
N.C. OFFICIAL FORM 20

I Am Renounced Freemason

Mail body:

Facebook: Aloha Sedona

HE SHOOK EUROPE AND REVEALED ISIS, PUTIN AND MERKEL: GRAND MASTER REVEALS WHO IS IN WHICH MASONIC LODGE!

Posted by [Joshua Flint](#) | Apr 17, 2016 | [Freedom Movement](#), [Mayors for De Jure Governance](#), [Top Stories](#) | 0 |

Rabbi Alicia Magal
Is Holding My Masonic
Apren At Synagogue
Sedona, Arizona.

Author: Z. K.

Tuesday, April 12, 2016 at 18:17

<http://www.dnevno.hr/planet-x/uздрмаво-европу-разоткло-исис-путин-меркел-велики-мвестар-откло-је-у-којој-масонској-љози-912786>

This is the first time in history that a grand master reveals the secret of Freemasonry – who is where. This just means the end of secrets and that evil does not hide anymore!

Earlier this year in Italy came out a scary book under the title “The Masons” in which in the simplest way is unmasked the whole conspiracy in which we find ourselves. The book has shocked the public because the Grand Master himself gave a list of people who govern and of those who have ruled the world and publicly revealed the names of their lodges from which can be read only one thing – they all together (and Putin included) are working on the same goal – the creation of the New world order, while the quasi-fight of ‘great powers’ is really just a play for the masses.

Grand Master Giele Magaldi also revealed a list of politicians and corporatist who, on the one hand, sit in the same lodges, but on the other hand, supposedly fight against each other.

For example, the leader of ISIS in the same Masonic lodge as for the example Tony Blair and Nicolas Sarkozy. Vladimir Putin sits in the same Masonic lodge with Angela Merkel, and he is, supposedly, fiercely fighting the New World Order and the Freemasons (so it is only shown). Grand Master and author of the book claims that the only aim of all politicians at the top of world governments is to establish the New World Order in which the elite will dominate the World.

Although the book made a real boom in Italy, all other world media simply went over that event, although there is nothing hidden. Check out who’s where in reemasonry. All the people on the list, says the author of the book, were crucial to the establishment of the New World Order, and were tasked by their lodges to perform tasks that go toward that goal, while they were in power.

Barack Obama (US President sits in a ‘Maat’ lodge. This lodge was founded by Zbigniew Brzezinski.

Vladimir Putin (Russian president sits in a Masonic lodge called ‘Golden Eurasia’).

Angela Merkel (German Chancellor) acted in the same lodge “Golden Eurasia”, now is a member of the “Valhalla”, “Parsifal” lodges).

Christine Lagarde (FMI director is the member of the Lodge “Three Eyes” and “Pan-Europa”).

George W. Bush (former President of the United States is in the Lodge “Hathor Pentalpha”).

Michael Leeden (US journalist and political expert is in a lodges, “White Eagle”, “Hathor Pentalpha”).

Condoleezza Rice (US politician was “Three Eyes”, “Hathor Pentalpha”).

Madeleine Albright (US politician was in “Three Eyes”, “Leviathan”).

Abu Bakr Al-Baghdadi (ISIS leader and the Islamic caliphate seats in the lodge ‘Hathor Pentalpha’).

Tony Blair (former UK Prime Minister acts in lodge ‘Edmund Burke,’ and then in ‘Hathor Pentalpha’).

David Cameron (also a former UK Prime Minister, is in a Lodge ‘Edmund Burke’ and ‘Geburah’).

Mariano Rajoy (former Prime Minister of Spain is in the Lodge “Pan-Europa”, then in the lodge “Valhalla”, and finally in the lodge “Parsifal”).

Antonis Samaras (former Minister of Greece flourishes in the lodge, “Three Eyes”).

Nicolas Sarkozy (politician and president of France from 2007 to 2012 was in the lodges, ‘Edmund Burke’, ‘Geburah’, ‘Atlantis-Aletheia,’ ‘Pan-Europa’, ‘Hathor Pentalpha’).

Manuel Valls (former French Prime Minister was the first in the lodge Grand Orient de France, then in ‘Edmund Burke’, ‘Compass Star-Rose / Rosa-Ventorum Stella’, ‘Der Ring’).

Bill Gates (corporatist sits in the “Star-Compass Rose / Rosa-Ventorum Stella”) lodge.

masonixxl

Mason of Masons – Masons have their own god

Albert Pike, the leading mason of the world in the book entitled “Morals and Dogma” better known as “Masonic Bible” describes how Masons not only hate “our” God but serve and worship devil, Lucifer. These are the quotes, or the instructions for the Masons which clearly states that the Baal whose temples are now erected worldwide is their deity, but that he is also the greatest enemy of God from the Bible.

– Every Masonic Lodge is a temple of religion and its teachings are religious instructions. At each stage of Masonry candidate seeks to reach the light. Masonry march and fight on the way to the light. The sun is an ancient symbol of generative power of the divine. Sun worship has become the basis of all religions of the ancient world. Sun is the hieroglyphic symbol of truth, because the source of Light. The sun, the all-seeing eye is in all of our lodges. A shining star represents a large central light that as sun was worshiped by so many nations as the true God, whom all Masons have to worship. Osiris himself is symbolized like a sun. And a god who presents itself as Adonai (the biblical word for only our lord god) God is a rival to Baal and Osiris.

Lucifer, light bearer! Strange and mysterious name of the spirit of Darkness. Lucifer, son of Dawn. Does he bring light on? You do not doubt this? – Pike writes in his book to his brothers Freemasons still stating atrocities – the devil, fallen Lucifer, or light bearer. Yes, Lucifer is God unfortunately for Adonai (he refers of our God).

The true and pure philosophical religion is the belief in Lucifer that is to Adonai. Lucifer is the god of light and goodness, who fights against

Adonai, the god of darkness and evil.

You do not understand what this is about? Here is what the Bible says:

“Woe to those who call evil good, and good evil. Who from darkness light make. That bitter make sweet and from sweet make bitter! “(Isaiah 5, 20).

Do you now understand why the world is being engulfed by evil?
masonixl

ABOUT THE AUTHOR

Joshua Flint

[Joshua Flint](#)

<https://thegoodlylawfulsociety.org/shook-europe-revealed-isis-putin-merkel-grand-master-reveals-masonic-lodge/>

Rabbi Alicia Magal
Sedona Synagogue
Arizona, USA





Enloe Medical Center (Main
campus)
1531 ESPLANADE
CHICO CA 95926

Chase Michael W
MRN: 345520, DOB: 6/29/1971, Sex: M
Adm: 2/7/2019, D/C: 2/11/2019



Scientists find 19 pieces of non-human DNA in the Human Genome.

According to a new study, eight percent of our DNA is ALIEN.

In fact, it is made up of NON-HUMAN, viral fragments.

The new study was published in the Proceedings of the National Academy of Sciences.

The recent study revealed that there is literally non-human DNA residing in modern humans' genome. This study comes after a group of researchers from Tufts and University of Michigan Medical School examined 2,500 people.

Experts discovered that our DNA is less human and that nineteen pieces of Ancient Viral DNA exist within our own genome.

Most strikingly, experts discovered the full genetic mockup for an entire virus within 2 percent of the people they examined. According to sciencedaily.com, whether or not the virus can be replicated or reproduced, is not yet known. But other studies of ancient virus DNA have shown it can affect the humans who carry it.

ScienceDaily reports that the study offers new insight on human endogenous retroviruses. HERV's are actually antique diseases which possess eerily similar characteristics to human immunodeficiency virus, the precursor to AIDS.

Experts believe that this 'Viral DNA0 has been passed down through thousands of generations of human beings. The study's authors are still unsure whether the ancient strains of DNA could cause infections.

"This one looks like it is capable of making infectious virus, which would be very exciting if true, as it would allow us to study a viral epidemic that took place long ago," says senior author and virologist John Coffin, Ph.D. of the Tufts University School of

Medicine. “This research provides important information necessary for understanding how retroviruses and humans have evolved together in relatively recent times.”

“Many studies have tried to link these endogenous viral elements to cancer and other diseases, but a major difficulty has been that we have not actually found all of them yet,” says co-first author Zachary H. Williams, a Ph.D. student at the Sackler School of Graduate Biomedical Sciences at Tufts University in Boston. “A lot of the most interesting elements are only found in a small percentage of people, which means you have to screen a large number of people to find them.”

“This is a thrilling discovery,” says co-first author Julia Wildschutte, Ph.D., who began the work as a Ph.D. student in Coffin’s lab at Tufts. “It will open up many doors to research. What is more, we have confirmed in this paper that we can use genomic data from multiple individuals compared to the reference human genome to detect new HERVs. But this has also shown us that some people carry insertions that we can not map back to the reference.”

Reference: <http://www.pnas.org/content/113/16/E2326.full.pdf>

Discovery of unfixed endogenous retrovirus insertions in diverse human populations

Julia Halo Wildschutte^{a,1}, Zachary H. Williams^{b,1}, Meagan Montesion^b, Ravi P. Subramanian^b, Jeffrey M. Kidd^{a,c}, and John M. Coffin^{b,2}

^aDepartment of Human Genetics, University of Michigan Medical School, Ann Arbor, MI 48109; ^bDepartment of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, MA 02111; and ^cDepartment of Computational Medicine and Bioinformatics, University of Michigan Medical School, Ann Arbor, MI 48109

Contributed by John M. Coffin, February 11, 2016 (sent for review November 25, 2015; reviewed by Norbert Bannert, Robert Belshaw, and Jack Lenz)

Endogenous retroviruses (ERVs) have contributed to more than 8% of the human genome. The majority of these elements lack function due to accumulated mutations or internal recombination resulting in a solitary (solo) LTR, although members of one group of human ERVs (HERVs), HERV-K, were recently active with members that remain nearly intact, a subset of which is present as insertionally polymorphic loci that include approximately full-length (2-LTR) and solo-LTR alleles in addition to the unoccupied site. Several 2-LTR insertions have intact reading frames in some or all genes that are expressed as functional proteins. These properties reflect the activity of HERV-K and suggest the existence of additional unique loci within humans. We sought to determine the extent to which other polymorphic insertions are present in humans, using sequenced genomes from the 1000 Genomes Project and a subset of the Human Genome Diversity Project panel. We report analysis of a total of 36 nonreference polymorphic HERV-K proviruses, including 19 newly reported loci, with insertion frequencies ranging from <0.0005 to >0.75 that varied by population. Targeted screening of individual loci identified three new unfixed 2-LTR proviruses within our set, including an intact provirus present at Xq21.33 in some individuals, with the potential for retained infectivity.

HERV-K | HML-2 | human endogenous retrovirus |
1000 Genomes Project | Human Genome Diversity Project

During a retrovirus infection, a DNA copy of the viral RNA genome is permanently integrated into the nuclear DNA of the host cell as a provirus. The provirus is flanked by short target site duplications (TSDs), and consists of an internal region encoding the genes for replication that is flanked by identical LTRs. Infection of cells contributing to the germ line may result in a provirus that is transmitted to progeny as an endogenous retrovirus (ERV), and may reach population fixation (1). Indeed, more than 8% of the human genome is recognizably of retroviral origin (2). The majority of human ERVs (HERVs) represent ancient events and lack function due to accumulated mutations or deletions, or from recombination leading to the formation of a solitary (solo) LTR; however, several HERVs have been coopted for physiological functions to the host (3).

The HERV-K (HML-2) proviruses (4–9), so-named for their use of a Lys tRNA primer and similarity to the mouse mammary tumor virus (human MMTV like) (10), represent an exception to the antiquity of most HERVs. HML-2 has contributed to at least 120 human-specific insertions, and population-based surveys indicate as many as 15 unfixed sites, including 11 loci with more or less full-length proviruses (5, 6, 8, 9). To distinguish the latter from recombinant solo-LTRs, we refer to these elements as “2-LTR” insertions throughout this study. The majority of these insertions are estimated to have occurred within the past ~2 My, the youngest after the appearance of anatomically modern humans (4, 8, 11). Population modeling has implied a relatively constant rate of HML-2 accumulation since the *Homo-Pan* divergence (5, 12, 13). All known insertionally polymorphic HML-2 proviruses have signatures of purifying selection, implying ongoing exogenous replication, and retain one or more ORFs

(8, 13–15). HML-2 expression has been observed in tumor-derived tissues as well as normal placenta in the form of RNAs, proteins, and noninfectious retrovirus-like particles (3, 16–19). These unique properties raise the possibility that some HML-2 group members are still capable of replication by exogenous transmission from rare intact proviruses, from the generation of infectious recombinants via copackaged viral RNAs, or from rare viruses still in circulation in some populations. A naturally occurring infectious provirus has yet to be observed, although the well-studied “K113” provirus, which is not in the GRCh37 (hg19) reference genome but maps to chr19:21,841,544, has intact ORFs (9) and engineered recombinant HML-2 proviruses are infectious in cell types, including human cells (20, 21). The goal of this study was to enhance our understanding of such elements by identifying and characterizing additional polymorphic HML-2 insertions in the population.

The wealth of available human whole-genome sequence (WGS) data should, in principle, provide the information needed to identify transposable elements (TEs), including proviruses, in the sequenced population. However, algorithms for routine analysis of short-read (e.g., Illumina) paired-end sequence data exclude reads that do not match the reference genome. Based on read

Significance

The human endogenous retrovirus (HERV) group HERV-K contains nearly intact and insertionally polymorphic integrations among humans, many of which code for viral proteins. Expression of such HERV-K proviruses occurs in tissues associated with cancers and autoimmune diseases, and in HIV-infected individuals, suggesting possible pathogenic effects. Proper characterization of these elements necessitates the discrimination of individual HERV-K loci; such studies are hampered by our incomplete catalog of HERV-K insertions, motivating the identification of additional HERV-K copies in humans. By examining >2,500 sequenced genomes, we have discovered 19 previously unidentified HERV-K insertions, including an intact provirus without apparent substitutions that would alter viral function, only the second such provirus described. Our results provide a basis for future studies of HERV evolution and implication for disease.

Author contributions: J.H.W., Z.H.W., J.M.K., and J.M.C. designed research; J.H.W., Z.H.W., M.M., and R.P.S. performed research; J.H.W., Z.H.W., M.M., and R.P.S. contributed new reagents/analytic tools; J.H.W., Z.H.W., R.P.S., J.M.K., and J.M.C. analyzed data; and J.H.W., Z.H.W., J.M.K., and J.M.C. wrote the paper.

Reviewers: N.B., Robert Koch Institute; R.B., University of Plymouth; and J.L., Albert Einstein Medical School.

The authors declare no conflict of interest.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. KU054242–KU054309).

See Commentary on page 4240.

¹J.H.W. and Z.H.W. contributed equally to this work.

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signatures stemming from such read pairs, specialized algorithms have been developed to detect TEs present within sequenced whole genomes. These methods seek to identify read pairs for which one read is mapped to a reference genome and the mate is aligned to the TE of interest (22). Additional criteria (e.g., read support, depth, presence of reads that cross the insertion junction) are then assessed to identify a confident call set. Recent applications of this general method to Illumina WGS data have indicated the presence of additional nonreference HML-2 insertions (12, 23), although validation and further characterization of these sites have been limited. Also, given the comparably short fragment lengths of typical Illumina libraries, it is not possible to distinguish between solo-LTR insertions and the presence of a 2-LTR provirus using these data alone, and experimentation is required to exclude sequencing artifacts.

To date, the number of human genomes analyzed for unfixed HML-2 proviruses is fairly small, limiting discovery of elements not present in the human reference genome, or “nonreference” elements, to those elements that are present in a relatively high proportion of individuals. Here, we build on existing detection methods to improve the efficiency of nonreference HML-2 identification from WGS data and assess the alleles present at each site. From analysis of more than 2,500 sequenced genomes, we have identified and characterized 36 nonreference insertions. We detected unique HML-2 insertions that were present in <0.05% to >75% of all samples and displayed variable presence across populations. Validation by locus-specific PCR confirmed three newly unreported 2-LTR proviruses within our dataset; one of these proviruses contains full ORFs for the viral *gag*, *pro*, *pol*, and *env* genes and lacks any obvious substitutions that would alter conserved sequence motifs, implying a potential for infectivity.

Materials and Methods

Data Analyzed. Illumina WGS data were obtained from 1000 Genomes Project (1KGP) samples, including a total of 2,484 individuals from 26 populations (24), and 53 individuals in seven populations from the Human Genome Diversity Project (HGDP) (25, 26). The 1KGP data were downloaded in aligned Binary Alignment/Map (BAM) format (<ftp://ftp.ncbi.nlm.nih.gov/1000genomes/ftp/data/>). HGDP data were processed as described (26), and are available at the National Center for Biotechnology Information (NCBI) Sequence Read Archive under accession SRP036155. Individual BAMs were merged using the Genome Analysis Toolkit (27) by population (1KGP) or dataset (HGDP). The 1KGP populations ranged from 66 to 113 individuals and had an effective coverage of ~1,067x ± 207.4x per pooled BAM; 53 HGDP samples were pooled to a single BAM of ~429x.

HML-2 Discovery from Read Pair Data. Candidate nonreference HML-2 LTRs were identified using RetroSeq (28). LTR-supporting read pairs were identified by running “discover” on individual BAM files, with read alignment to the HML-2 LTR5Hs consensus elements from RepBase (29) and previous reports (20, 21). RepeatMasker (30) HERV coordinates from the GRCh37/hg19 reference were used for exclusion of previously annotated sites. RetroSeq “call” was applied to the merged BAMs (above), requiring a read support of ≥2 for a call. A maximum read depth per call of 10,000 was applied for the increased coverage of the BAMs. To capture only novel insertions, calls within 500 bp of an annotated HML-2 LTR were excluded. Other RetroSeq options were kept at default values.

Reconstruction of Viral-Genome Junctions. For each RetroSeq candidate call, supporting read pairs and split reads within 200 bp of the assigned break were extracted from each sample and subjected to de novo assembly using CAP3 (31, 32). Assembled contigs were subjected to RepeatMasker analysis to confirm the LTR presence and type (i.e., LTR5Hs) (30), and then filtered to identify the most likely candidates, requiring separate contigs that contained the respective 5' and 3' HML-2 LTR edges, and the presence of ≥30 bp of both the LTR-derived and genomic sequence at each breakpoint. We examined contig pairs for the presence of 4-bp to 6-bp putative TSDs, but did not require their presence for a call. Output assemblies were aligned to the hg19 reference to confirm the position of the preintegration, or empty, site per call.

Analysis of Unmapped Reads for LTR Junction Discovery. Unmapped reads were retrieved from BAM files with Samtools (<samtools.sourceforge.net>) from all 53 HGDP samples and 825 1KGP samples (≥10 samples per 1KGP population) and searched for a sequence that matched the 5' HML-2 LTR edge (TGTGGGGAAAGCAAGAGA), 3' LTR edge (GGGGCAACCCACCCA-TACA), or 3' LTR variant (GGGGCAACCCACCCATTCA) that is observed in a subset of human-specific elements, requiring ≥10 bp of non-LTR sequence per read. Reads matching reference HML-2 junctions were removed. Candidate reads were then aligned to the hg19 reference to identify genomic position. Sequences with no match to hg19, with <90% identity, or that aligned to gaps or multiple genomic positions were searched against the chimpanzee (*panTro4*) and gorilla (*gorGor3*) references, and available human WGS data from the NCBI Trace Archive to identify insertions in structurally variable regions.

Validation and Sequencing. DNA from samples yielding positive reads was obtained from Coriell or the Foundation Jean Dausset-Centre d’Étude du Polymorphisme Humain. Coordinates for each insertion were based on mapping of assembled contigs or read-captured flanking sequence to the hg19 reference. PCR was performed with 100 ng of genomic DNA using primers flanking each site to detect either the empty site or solo-LTR alleles. A separate PCR was run to infer a 2-LTR allele with a primer situated in the HML-2 5' UTR paired with a flanking primer (6, 8, 33). Capillary sequencing was performed on at least one positive sample. The 2-LTR alleles were amplified in overlapping fragments from a single sample and sequenced to ≥4x (8, 9), and a consensus then constructed with the read traces from each site. Complete sequences are available in the NCBI GenBank under accession nos. KU054242-KU054309.

Phylogenetic Analysis. Full-length sequences representing either solo-LTRs or proviral 5' and 3' LTRs were aligned to the consensus HML-2 using ClustalX (34); the alignment was then edited, and truncated LTRs were removed (8). A single neighbor-joining tree was generated from the remaining 68 insertions (90 total LTRs) using MEGA6 (35). The Kimura 2 parameter model was used for branch length estimation, with α of 2.5 and deletions treated pairwise. Support for the tree was assessed using 1,000 bootstrap replicates.

Age Estimations. LTR divergence was used to infer the time since insertion, normalized to a neutral mutation rate of 0.24–0.45% per My as measured by calculating the divergence between orthologous human and chimpanzee HML-2 proviruses (6, 8). Alternatively, using mutation rates directly obtained from pedigrees (36) results in estimated times of insertion fourfold to ninefold as old and implausible integration times for many proviruses. Briefly, the nucleotide differences were totaled between proviral 5' and 3' LTRs, and the total was divided by the LTR nucleotide length. The percentage of divergence was then divided by the upper and lower bound mutation rates for age range estimation (million years) (6, 8).

In Silico Genotyping. Genotyping was performed for both reference (hg19) and nonreference unfixed insertions using a read-based method that has been used for genotyping Alu TEs in humans (32, 37). Briefly, discrete reference (e.g., the empty state) and alternate (e.g., the insertion state) alleles were recreated for each locus, including ±600 bp upstream and downstream of the insertion point based on hg19 coordinates. Within those coordinates, Illumina read pairs that had at least one read mapped to the empty allele were extracted for each site (requiring a mapping quality score >20). Treating the reconstructed alleles as the target genome, genotype likelihoods were then determined based on remapping of those reads to either allele, with error probabilities based on read mapping quality as described previously (32, 38). Samples without reads aligning to the reconstructed reference and alternate alleles for a particular site were not genotyped at that site. Insertion allele frequencies were estimated per site for all genotyped samples as the total number of insertion alleles divided by the total number of alleles. Detection frequencies (the proportion of individuals carrying the insertion) were calculated as the number of individuals with the insertion divided by the total number of individuals genotyped at each locus. We note that the reference insertion at 7p22.1, which is present as a tandem duplication of two proviruses that share a central LTR (6, 15), was treated as a single insertion (chr7:4,622,057–4,640,031). Nine of the 36 nonreference loci could not be aligned to the hg19 reference and were excluded from genotyping: insertions within duplicated segments (we refer to these as dup1 through dup4), insertions of unusual assembled structure (10q24.2b and 15q13.1), or insertions that could not be mapped to the hg19 assembly (10q26.3, 12q24.32, and 22q11.23b).

Proportion of Provirus Carriers. Unique 30-mers were identified from a set of 51 reference and nonreference elements from the HML2 subgroup using Jellyfish (39). Candidate 30-mers were further mapped against the GRCh37 genome reference using mrsFAST and mrFAST (40), and k-mers with >100 matches within an edit distance of two were omitted, resulting in a set of 83,343 k-mers. The position of each 30-mer in each HML2 element was determined, and 1,445 k-mers that crossed LTR–internal proviral junctions were omitted, leaving 5,698 k-mers that were unique to an LTR and 76,200 k-mers that were unique to the internal sequence. Total observed counts for each k-mer were determined in WGS sequence data from 53 HGDP and 2,453 1KP samples. The median k-mer depth for each element in each sample was determined. Median depths were normalized per sample by dividing by the maximum median depth observed for a proviral sequence. Elements with a normalized median k-mer depth ≥ 0.25 were considered to be present in a sample. The proportion of individuals for which an element was present was then determined for each population.

Results

HERV-K (HML-2) Insertions Discovered from WGS Data. The goal of this study was to use the extensive available WGS data in the 1KGP and HGDP collections to identify relatively rare polymorphic nonreference HML-2 insertions. To make the fullest use of all sequence information available within these data, we applied two approaches to identify candidate nonreference HML-2 insertions in the raw reads for these collections (Fig. 1).

First, we identified insertions based on read pair signatures using the program RetroSeq (28) (Fig. 1, Left). To improve the detection of insertions present in multiple samples, we combined reads within a population (1KGP) or study (HGDP) (32). Excluding calls within ± 500 bp of a reference HML-2 sequence, we obtained 140.3 ± 56.1 candidate calls per pool. Next, we applied

a de novo assembly approach to insertion-supporting reads to reconstruct the LTR–genome junction for as many sites as possible (32). Given the size of HML-2 LTRs (~968 bp per LTR), we inferred the presence of an insertion based on the presence of separately assembled 5' and 3' breakpoints. This requirement reduced false-positive calls, for example, as caused by SVA elements (SINE-VNTR-Alu), which have high identity to bases 1–329 of the HML-2 LTR. A total of 29 candidate HML-2 insertions with a flanking sequence were assembled, including K113 (19p12b; also see Fig. S1 A and B and Table 1).

As a second approach, we mined unmapped reads for evidence of LTR–genome junctions captured in reads that could not be placed on the human reference (Fig. 1, Right) and would therefore be missed using current read-based detection methods, such as RetroSeq. Using this approach permitted the identification of insertions in regions absent from the human reference. Excluding reads that could be aligned to annotated HML-2 junctions, we obtained overlap for the 29 candidate sites identified above, as well for as seven loci not found in assembled RetroSeq calls (Fig. S1C and Table 1). Our final call set includes 17 insertions identified in recent reports from Marchi et al. (12) and Lee et al. (41). The nomenclature for all sites is as maintained in those studies and in other previous reports (8, 33).

Validation and Sequencing. We validated the presence of 34 of the 36 candidate insertions in at least one individual predicted to have the insertion (Table 1 and Dataset S1). The remaining two sites (at 10q24.2 and 15q13.1) were predicted to have an unusual inverted repeat structure based on assemblies of supporting reads at either site (Fig. S2), and could not be conclusively confirmed by sequencing, possibly due to hairpin formation. For the 34 validated nonreference sites, we confirmed 29 sites as having solo-LTRs and five sites with 2-LTR proviruses (at 8q24.3c, 19p12d, 19p12e, Xq21.33, and the published K113 provirus at 19p12b; also see Table 1). Four of the solo-LTRs were situated within duplicated segments and could not be mapped to unique positions in the hg19 reference (dup 1–dup 4), and two insertions, at 12q24.32 and 10q26.3, were located within structurally variable regions that are absent from the hg19 reference (Fig. S3). One insertion was initially mapped to the reported 9q34.11 locus (12, 41); however, comparison of the Sanger reads from its validated LTR–genome junctions revealed unexpectedly low identity in the extended flanking sequence. Our reexamination of this site indicates it maps instead to a region that is not in hg19 but is present in an alternate scaffold in the GRCh38 assembly at 22q11.23 (Table 1). This discrepancy may explain why this particular site has only been previously inferred by reads supporting only the 5' breakpoint of the integration (12, 41, 42).

We obtained full sequences for 30 of the 36 candidate insertions in at least one individual predicted to have the insertion (Dataset S1); these sequences included the full-length insertion at Xq21.33 that was found to have intact viral ORFs (NCBI GenBank accession no. KU054272). The remaining six insertions were extracted or reconstructed from public sequence databases for subsequent analysis as follows. The full sequence from one locus identified within a duplicated segment was reconstructed from Sanger reads corresponding to that site from the NCBI Trace Archive (dup1). The sequence flanking the insertion at 12q24.32 could be mapped to a previously sequenced fosmid clone in a region corresponding to an encompassing deletion of ~14.3 kb in the hg19 reference (43) (Fig. S3A). Another insertion, corresponding to a 2-LTR provirus, was also from a sequenced fosmid clone (19p12d) as reported (41, 44). The complete sequence of the K113 provirus (19p12b) was from the GenBank (accession no. AY037928). One solo-LTR, 1p31.1c, was detected and validated as a solo-LTR in a single individual of the 1KGP Yoruba. We searched for, but did not find evidence of, this site in subsequent PCR screens of other samples.

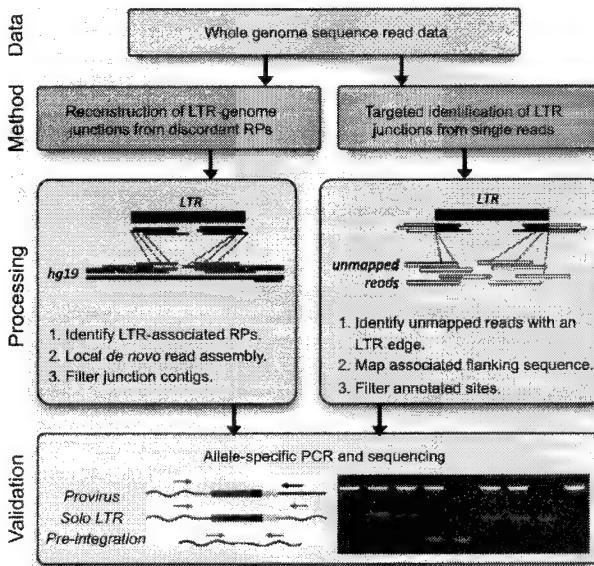


Fig. 1. Approaches for the detection of nonreference HML-2 insertions from WGS read data. Illumina short reads were processed by one of two methods. (Left) Read pairs (RPs) were identified that have one read mapped to the genome (gray) and mate to reads that map to the sequence matching the HML-2 LTR consensus (black). Supporting reads from each site were extracted and subjected to local assembly, and the resulting contigs were analyzed for the presence of LTR–genome junctions. (Right) Unmapped reads from each sample were identified that contained a sequence corresponding to the LTR edge, and the cognate sequence was then used to determine candidate integration positions from genomic data. (Bottom) PCR and capillary sequencing were used to validate candidate insertions in reactions that used flanking primers (gray arrows) to detect the presence of a solo-LTR or empty site, or a flanking primer paired with an internal proviral primer (black arrow) to infer the presence of a full-length allele. Representative products are shown in a genotyping gel to the right.

Table 1. Nonreference HML-2 insertions in human genomes

Locus	Coordinate GRCh37/hg19	Alias*	Alleles [†]	Flanking region and other properties	First report in humans (source)
1p13.2 [‡]	chr1:111,802,592	De5;K1	LTR, pre	L1 (L1PA6)	(41)
1p21.1 [‡]	chr1:106,015,875		LTR, pre		(12)
1p31.1c	chr1:79,792,629		LTR, pre	AluSz	This study
1q41	chr1:223,578,304	K2	LTR, pre	L1 (L1MDa)	(12)
3q11.2	chr3:94,943,488		LTR, pre	L1 (L1PA10)	This study
4p16c	chr4:9,603,240	K6	LTR, pre	ERV1 (HERVS71)	(12)
4p16d	chr4:9,981,605		LTR, pre	L2 L2b; SLCA29 intron 5/6	This study
5p15.32	chr5:4,537,604		LTR, pre	ERV1 (LTR1C)	This study
5q12.3	chr5:64,388,440	Ne7;K12	LTR, pre	L1 L1M6	(12)
5q14.1	chr5:80,442,266	De6/Ne1;K10	LTR, pre	RASGRF2 intron 17	(12)
6p21.32	chr6:32,648,036		LTR, pre	L1 (L1PA10)	(12)
6p22.3	chr6:16,004,859		LTR, pre	AluSx	This study
6q26	chr6:161,270,899	De2;K12	LTR, pre		(12)
7q36.3	chr7:158,773,385		LTR, pre		This study
8q24.3c	chr8:146,086,169		pro, pre	ERV1 (LTR46); COMMDS5 intron 9 of transcript variant 2; gag and pro ORFs	This study
10q24.2b	chr10:101,016,122	De12		ERV1 MalR (MSTD); unexpected structure	This study
10q26.3 [§]	chr10:134,444,012		LTR, pre	INPP5A intron 2	This study
11q12.2	chr11:60,449,890	De4;K18	LTR, pre	L1 (L1M4); LINC00301 intron 6	(12)
12q12	chr12:44,313,657	Ne6;K20	LTR, pre	L1 (L1MB1); TMEM117 intron 2	(12)
12q24.31	chr12:124,066,477	K21	LTR, pre	AluSx1; LOC101927415 exon 3	(12)
12q24.32 [§]	chr12:127,638,080–127,639,871		LTR, pre	ERV1 (MER57); deleted in hg19; from fosmid CloneDB: AC195745.1 bases 17648–18615	(43)
13q31.3	chr13:90,743,183	Ne2;K22	LTR, pre	SINE (FLAM_A); LINC0559 intron 3	(12)
15q13.1	chr15:28,430,088			HERC2 intron 56; unexpected structure	This study
15q22.2	chr15:63,374,594	K24	LTR, pre		(12)
19p12b	chr19:21,841,536	De1;K113	pro, pre		(9)
19p12d	chr19:22,414,379		pro, pre	Deletion in 5' LTR; pro ORF; insertion within fosmid clone accession AC245253.1	(41)
19p12e	chr19:22,457,244	De11	pro, pre	AluSq	This study
19q12 [§]	chr19:29,855,781	De3;K28	LTR, pre	LOC284395 intron 9	(12)
19q13.43	chr19:57,996,939	Ne5	LTR, pre	2 kb upstream of ZNF419	This study
20p12.1	chr20:12,402,387	De14 [*] ;K30	LTR, pre		(12)
22q11.23b [§]	chr22:23852639–23852640	De7;K16	LTR, pre	ERVL-MalR (MLT1C); maps to Hg38 alt locus scaffold_22_K1270878v1_alt:156355–180653	This study
Xq21.33	chrX:93,606,603	De9	pro, pre	L1 (L1MD1); gag, prol, pol, env ORFs	This study
Dup 1 [§]	Not determined		LTR	Flank maps to centromere associated duplications on multiple chromosomes	This study
Dup 2 [§]	Not determined		LTR, pre	Flank maps to duplicated regions within predicted FAM86 and ALG1L2 exonic variants	This study
Dup 3 [§]	Not determined		LTR, pre	Flank maps to 3 segmental duplications on chr1	This study
Dup 4 [§]	Not determined		LTR, pre	Deletion in hg19 reference; putative empty site on chr19 within fosmid CloneDB: AC232224.2	This study

*Reported originally in the sequenced Neandertal (Ne) or Denisovan (De) by Agoni et al. (42) or Lee et al. (51), or in modern humans (K) by Marchi et al. (12) or Lee et al. (41).

[†]Alleles detected. LTR, solo LTR; pre, preinsertion site; pro, 2-LTR provirus.

^{*}Previously PCR validated as solo-LTR by Lee et al. (41).

[‡]Insertion is located within an encompassing structural variant not present in the hg19 reference.

Estimated Frequencies of Unfixed HML-2 Loci. We performed in silico read-based genotyping to obtain estimations of the allele frequencies of 27 nonreference insertions with clear integration coordinates, and extended the analysis to include 13 annotated polymorphic HML-2 loci from the hg19 human reference (5, 8) (Dataset S2). Briefly, reference and alternate alleles representing each HML-2 locus were recreated, and individual genotypes were then inferred based on the remapping of proximal Illumina reads to the reconstructed alleles per site per sample (*Materials and Methods*). Given the larger size of the HML-2 LTR (~968 bp) and relatively short reads in these data, 2-LTR and solo-LTR insertions are indistinguishable in read-based genotyping alone, such that genotypes were based on the presence or absence of

the HML-2 insertion at each locus. Values reported below correspond to allele frequencies unless otherwise noted.

Estimated frequencies of the variable HML-2 insertions present in the reference genome ranged from ~0.25 to >0.99 in genotyped samples (Fig. 2, *Upper*). Sites with the highest estimated frequencies corresponded to those loci previously reported with a solo-LTR or provirus present, but not a preinsertion site, based on limited PCR screens of those sites (6) (at 1p31.1, 3q13.2, 7p22.1, 12q14.1, and 6q14.1 in Fig. 2). This pattern is consistent with variability at these sites based predominantly on the 2-LTR and solo-LTR states. Genotyping of the insertions at 11q22.1 and 8p23.1a (K115) implied the presence of both insertion and preinsertion alleles, also consistent with PCR screens in other reports (6, 9, 33, 45),

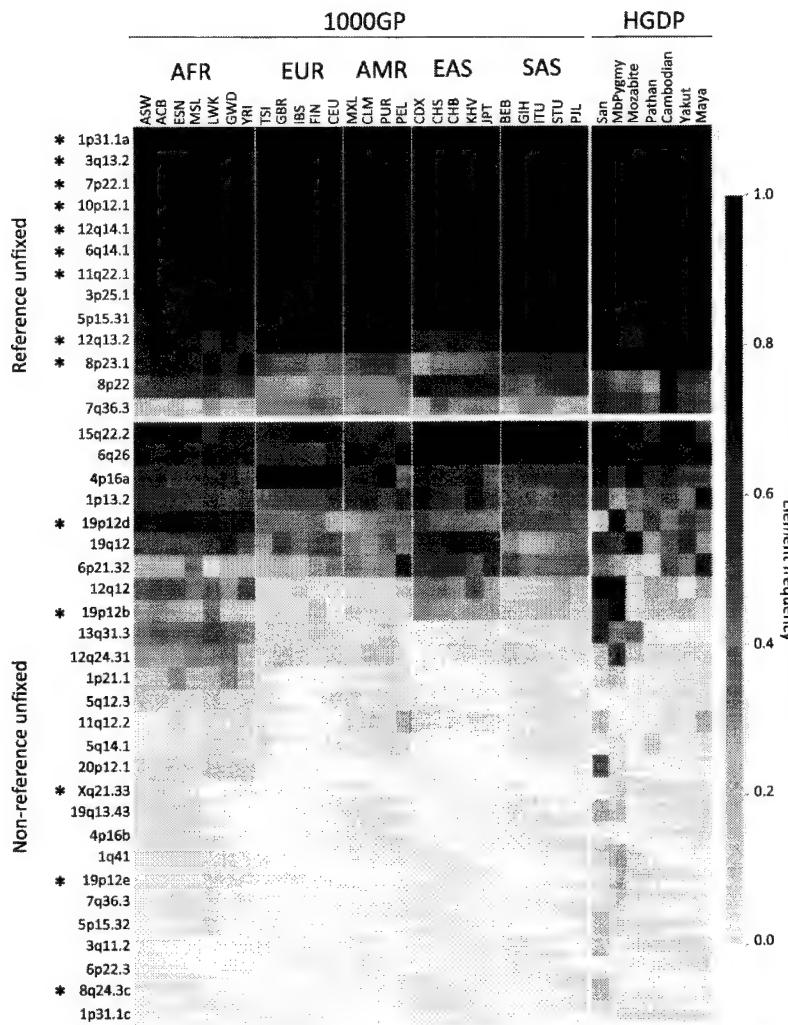


Fig. 2. Estimated insertion allele frequencies of unfixed HML-2 insertions in humans. A total of 40 HML-2 loci were subjected to *in silico* genotyping: 13 sites represented the unfixed HML-2 loci from the hg19 reference, and 27 sites corresponded to nonreference polymorphic HML-2 reported here. Genotypes were inferred for each unfixed HML-2 locus across samples based on remapping of Illumina reads to reconstructed insertion or empty alleles corresponding to each site. Samples lacking remapped reads at a particular site were excluded from genotyping at that site. Allele frequencies were then calculated for each population as the total number of insertion alleles divided by total alleles. Allele frequencies are depicted as a heat map according to the color legend to the right. The 1KGP (1000GP) and HGDP populations are labeled above (also refer to Dataset S1 for population descriptors and other information). The locus of each of the unfixed HML-2 loci is labeled to the left according to its cytoband position. An asterisk is used to indicate insertions that have confirmed full-length copies. (*Upper*) Estimated distribution of reference unfixed HML-2 [from loci reported by Subramanian et al. (11) and Belshaw et al. (5)]. (*Lower*) Estimated distribution of nonreference HML-2 insertions. AFR, African; AMR, Admixed American; EAS, East Asian; EUR, European; SAS, South Asian.

noting the higher frequency of K115 within our samples (~53%) than in those reports (up to ~34% depending on ancestry). Four unfixed reference solo-LTRs ranged in frequencies from ~0.25 to as high as ~0.93, also consistent with previous analysis of these sites (5). Extending the analysis to the 85 remaining human-specific HML-2 insertions that are suitable for genotyping in the human reference (81 solo-LTRs and four full-length proviruses) (5, 8) was consistent with sample-wide fixation among the vast majority of these loci; just eight loci had evidence of the nonreference allele among genotyped samples (Fig. S4 and Dataset S3).

Estimated frequencies of the nonreference HML-2 insertions were inferred to be from <0.0005 (the insertion having clear support in one or few individuals) to >0.75 of genotyped samples (Fig. 2, *Lower*). More than half of the nonreference HML-2 insertions were rare, with 15 insertions detected at frequencies of <5% and six insertions in <1% of all samples; just four of these loci have been previously reported (12). Sites with the lowest allele frequencies were predominantly in individuals of African

ancestry, with nine of 13 loci inferred in <5% of all samples but mostly limited to African populations, although insertions were also detected in non-African samples at ~0.005 to ~0.016 in those populations (e.g., at 5q14.1 and Xq21.33 in Fig. 3). The solo-LTR insertion at 1p31.1c was only identified in a single sample and was not detected in any other sample by genotyping; however, this observation does not exclude the possibility of its presence in some individuals, given the variability in read coverage between samples (*Discussion*). Nine of the 10 common insertions (detected in >10% of all samples), including the K113 provirus, have been previously reported in searches of WGS data (12, 41). A comparison of the overall presence of each HML-2 insertion, calculated as the proportion of individuals with evidence of the insertion, was generally in agreement with those reports (Fig. S5). The presence of K113 was estimated at a higher prevalence across samples here than in previous reports, in ~27% of all samples and as high as ~52% in African

populations, consistent with the prevalence of this insertion varying with ancestry (45).

Because the above analysis cannot distinguish between solo-LTR and 2-LTR alleles, we used a k-mer counting approach to infer the presence of 2-LTR HML-2 sequences in each sample. WGS data from each sample were queried using a catalog of 30-mers unique to each proviral sequence. Elements with a normalized depth ≥ 0.25 were inferred to be present in a sample. We find large variation in the proportion of individuals estimated to carry 2-LTR alleles for different elements, with 2-LTR alleles for elements present in the reference genome detected in a high proportion of individuals, whereas the nonreference 2-LTR insertions discovered in this study are extremely rare (Fig. S6 and Dataset S3). For example, we identified 44 samples with k-mer counts consistent with the presence of a 2-LTR allele for the Xq21.33 insertion.

LTR-Based Analysis of Unfixed HML-2 Proviruses. Using sequence information obtained for each insertion, we performed an LTR-based phylogenetic analysis (Fig. 3). Because proviral LTRs are identical at integration, the two LTRs on the same provirus will always pair in a phylogenetic tree, barring recombination between elements (46). Their unique source is further supported by the presence of TSDs, which are preserved during solo-LTR formation (6, 46, 47). To create the most informative tree, we added the LTRs from 21 human-specific proviruses, including 11 polymorphic 2-LTR insertions as reported by Subramanian et al. (8) and four unfixed solo-LTRs as reported by Belshaw et al. (5), to our validated set of LTRs from three 2-LTR proviruses (insertions at 8q24.3c, 19p12e, and Xq21.33), the 3' LTR of a truncated provirus (19p12d), and 30 solo-LTRs.

The analysis revealed a major lineage leading to a well-supported clade that contained all human-specific and polymorphic HML-2 sequences (Fig. 3A, boxed); a minor lineage included HML-2 elements that are fixed in humans (8) and did not contain any newly identified insertions. This phylogeny is consistent with previous analyses (8, 13, 46); however, the addition of our 34 unfixed loci permitted a more detailed examination of variable insertions (Fig. 3B). The majority of unfixed insertions clustered with the HML-2 consensus LTR (● in Fig. 3B) in a clade (*) in Fig. 3B whose members tended to have the shortest branches, consistent with their relatively recent integration and insertionally variable presence within humans (also refer to filled boxes in Fig. 3B). The human-specific reference elements were also distributed within this clade, consistent with previous observations (8). The remaining insertions were on branches with longer lengths, reflecting changes that accrued before insertion as well as during their longer existence as endogenous elements. We searched for, but did not observe, mispaired LTRs from the 2-LTR proviruses reported here. Subsequent examination of the TSDs from these proviruses confirmed all were intact, indicating these elements have not seeded past rearrangements (46). Extending this comparison with the TSDs of the identified solo-LTRs also verified their intact state.

Properties of Nonreference 2-LTR HML-2 Integrations. Assuming a constant mutation rate, the nucleotide divergence between cognate 5'-3' LTR pairs may be used to estimate the time since integration (47). Using this method, we previously estimated the average age of the human-specific 2-LTR insertions to within $\sim 2.7 (\pm 1.1)$ My (8). Applying this method to the 2-LTR proviruses identified here suggests these insertions were formed within ~ 0.67 – 1.8 Mya (Table 1). Further refinement for the youngest elements is limited, because the variance for age estimates increases significantly for insertions with very little or no LTR divergence. The 19p12d provirus was excluded from this analysis due to deletion of the majority of its 5' LTR (Fig. 4). This truncation has also been observed in a few reference LTR5Hs

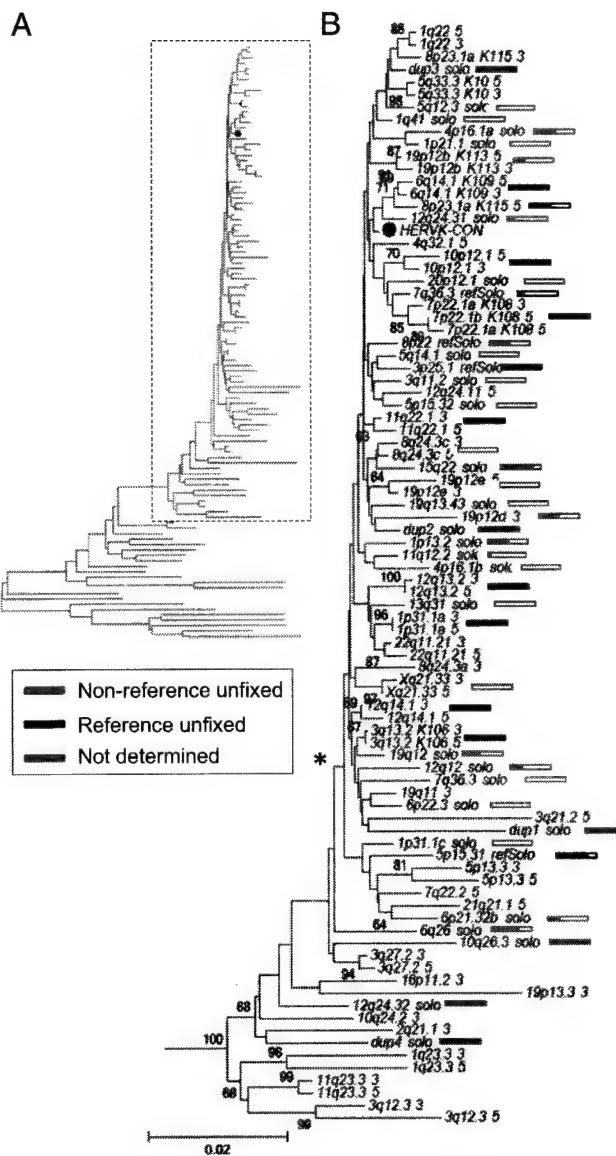


Fig. 3. Phylogenetic construction of HML-2 LTRs within humans. (A) Neighbor-joining tree was constructed based on the aligned nucleotide sequences corresponding to HML-2 LTRs from the LTR5Hs group, specifically including those nucleotide sequences considered to be human-specific and/or polymorphic. The LTRs were extracted from (i) all reference HML-2 proviruses previously inferred as belonging to the LTR5Hs HML-2 subgroup [as reported by Subramanian et al. (11)], (ii) unfixed reference solo-LTRs [as reported by Belshaw et al. (5)], and (iii) unfixed nonreference insertions as reported here. Both 5' and 3' LTRs were used for full-length insertions, when present. The closed circle (●) indicates the taxon corresponding to the HERV-K_{CON} LTR within the tree. Classic nomenclature has been included in taxon names for the better studied insertions: K113 (19p12b), K108 (7p22.1), K115 (8p23.1a), K106 (3q13.2), and K109 (6q14.1). (B) Detailed view of branches representing unfixed HML-2 insertions. Individual HML-2 loci are indicated for each branch as follows: the cytoband followed by a 5' or 3' for the 5' or 3' LTRs from full-length insertions, solo for nonreference unfixed solo-LTR insertions, or refSolo for reference unfixed loci. An asterisk is used to indicate the position of the clade containing the majority of unfixed insertions. Boxes are used to indicate estimated allele frequencies for each unfixed insertion at the end of each respective branch. The filled area within each box is shown as proportional to the estimated frequency of the insertion in all samples; the derived values are provided in Dataset S3. Gold and black boxes are used to represent nonreference and reference unfixed insertions, and gray bars indicate the elements for which the frequency could not be determined.

2-LTR insertions (8q24.3a, 10q24.2a, and 19q11) (8), which all possess unique, intact TSDs and do not share any flanking sequence, supporting their classification as independent integrations. It is likely that this common rearrangement occurred as a result of aberrant strand transfer during RT, as has been discussed (48).

HML-2 proviruses are classified by the presence or absence of a 292-bp deletion at the *pol-env* boundary, designated “type 1” or “type 2,” respectively. Deletion of a splice site in type 1 elements obliterates *env* and *rec* expression and results in mRNA encoding an ~9-kD protein, Np9, of possible cellular function (17, 19, 49). Sequence comparison of the 2-LTR proviruses identified here classifies the 19p12d and 19p12e elements as type 1 and 8q24.3c and Xq21.33 as type 2. The 19p12d and 19p12e insertions were found to have intact *pro* and *gag* ORFs, respectively, and the 8q24.3c insertion had both *gag* and *pro* ORFs. The Xq21.33 2-LTR element was found to be intact with ORFs for all HML-2 encoded genes (i.e., *gag*, *pro*, *pol*, *env*, *rec*) (Fig. 4). Indeed, it differs by only 39 of a total of 2,820 amino acids (98.6% amino acid identity) in all genes from the infectious consensus provirus HERV-K_{CON} (21). We searched for, but did not observe, any substitutions that would alter conserved sequence motifs (including the YIDD motif in reverse transcriptase), making this element a candidate for activity. This provirus is only the second naturally occurring intact HERV to be described, with the other being the noninfectious K113 (19p12b) that shares 98.9% amino acid identity to HERV-K_{CON} (9). Its potential for generation of infectious virus is currently under investigation.

Discussion

We report 36 nonreference HML-2 insertions, including 19 previously identified loci, from analysis of WGS read data from more than 2,500 globally sampled individuals. Seventeen of the 36 sites were recently reported in humans (12, 41), although with limited validation or element characterization. Here, we take full advantage of the 1KGP and HGDP WGS read data to identify nonreference viral-genome junctions from assembled anchored read pairs and individual unmapped reads, and use these data to estimate the presence of each of these elements within our sampled populations. We validated the presence of 34 of the 36 loci, including five loci with 2-LTR proviruses (including K113) and 29 solo-LTRs, and report the complete sequences for 30 of these insertions, including a 2-LTR provirus at Xq21.33 that appears to be intact. We provide a thorough analysis of unfixed HML-2 insertions that complements and builds on previous studies, and should enable future examination of the HML-2 group.

We used the available reads from each sample for *in silico* genotyping of a subset of sites to infer the population-wide frequencies of unfixed HML-2 elements, which is impractical on this scale in standard PCR-based screens. The inferred allele frequencies of the nonreference insertions ranged from 0.05 to >75% of genotyped samples and varied between populations, generally with the highest presence in African populations. With the exception of two previously sequenced sites in our set (dup1 and 12q24.11), all nonreference insertions were validated in samples of African ancestry, as has been observed for all HERV-K loci characterized to date, implying their insertion before the human migration out of Africa ~45,000–60,000 y ago (50). These two insertions could not be confidently mapped to the hg19 reference, and were therefore excluded from genotyping. All but one nonreference insertion was identified in more than one individual, with the exception being the 1p31.1c solo-LTR validated in NA18867. Genotyping of that site failed to reveal (but does rule out) its presence in other individuals. Analysis of the surrounding region revealed the presence of several SNPs that were unique to NA18867 within the 1KGP panel, suggesting that 1p31.1c may be associated with a very rare haplotype, rather than a de novo event, in the absence of comprehensive screening. These observations support the utility of short read data for

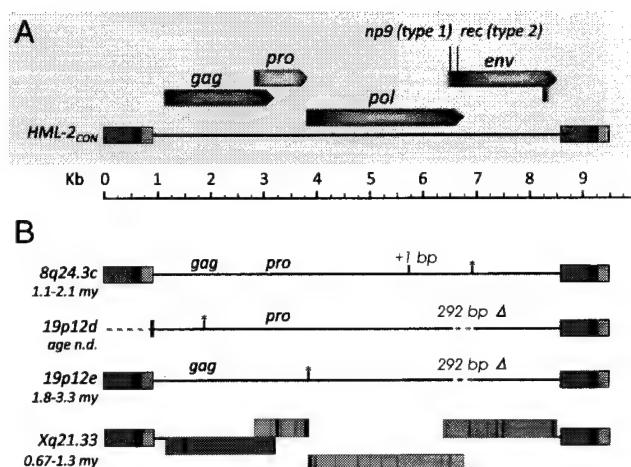


Fig. 4. Features of newly identified HML-2 proviruses in humans. (A) Schematic representation of the consensus HML-2 provirus, including the viral gene positions and frames to scale. Splice sites for np9 (type 1 insertion, 292 bp Δ) and rec (type 2) are indicated. Regions within the LTRs are colored in gray: U3, medium; R, dark; U5, light. (B) Features of nonreference identified proviruses are shown to scale. The region of 292 bp is labeled for type 1 insertions. Age estimations are shown for each site. n.d., not determined. The black vertical line indicates a frameshift mutation (as indicated “+1 bp”); black lines with asterisks are used to indicate positions of stop codons where present. Reading frames are shown for the Xq21.33 2-LTR provirus as colored as in A. Black vertical lines within the frames indicate the positions of base changes that are observed in other full-length HML-2 proviruses. Red vertical lines are used to indicate base changes that are unique to the sequenced Xq21.33 provirus.

element discoveries and sequence-based analysis, but also underscore the necessity of additional experimental validation steps and characterization of candidate proviruses.

Eight of our validated loci have been recently reported in the genomes of two sequenced archaic samples (42, 51) in addition to modern humans (12). We confirmed an additional three reported “archaic” sites in our data [19p12e and 10q24.2b, respectively: “De11” and “De12” in the study by Agoni et al. (42); 19q13.43: “Ne5” in the study by Lee et al. (51)], but found no evidence of the remaining eight reported archaic events. Properties of these 11 HML-2 loci are more consistent with insertion before the most recent ancestor with modern humans ~0.6–0.8 Mya (52) than with introgression. For example, the 2-LTR insertions at 19p12e and Xq21.33 are most prevalent in samples of African ancestry, and LTR divergences indicate their respective insertions to have been ~1.8–3.3 Mya and ~0.67–1.3 Mya, consistent with this time frame. Both sites are rare, with sample-wide allele frequencies estimated at 0.0103 and 0.0157 (~0.026–0.069 in the African sample) in our data (Dataset S3). Of the remaining genotyped loci also in archaic genomes, each was also most represented in African ancestry, with exception of the insertions at 11q12.2 and 5q14.1 (sample-wide allele frequencies estimated at ~0.046 and 0.026) that appeared most frequently in populations from the Americas or of East Asian ancestries but are also present in African populations, again implying ancient events (50). Given their overall distribution, it is likely these insertions are also older, although our ability to estimate insertion times is limited, given their presence as solo-LTRs.

We confirmed the presence of full-length proviruses at four loci, including the Xq21.33 provirus, which appears to be intact and without obvious defects, which implies the potential for replication competence and is now under further investigation. Given a genomic mutation rate of $\sim 2.2 \times 10^{-9}$ changes per site per year (53), an ERV could maintain infectivity over very long periods, and a number of infectious ERVs are known in other

species, including mice, cats, and some birds (3). Although such elements are likely to be regulated by silencing and downstream host mechanisms, disease states causing prolonged reactivation of HERVs could drive expression of such proviruses or the generation of recombinant infectious chimeras, as has been shown to occur in Ab-deficient mice (54) and as claimed for HML-2-derived transcripts in the blood of HIV-infected individuals (44). Indeed, a “recombinant” HML-2 provirus engineered from just three well-studied defective reference loci is infectious (derived from portions of K109, K115, and K108, respectively, at 6q14.1, 8p23.1a, and 7p22.1) (20), as are the HML-2 consensus genomes (20, 21). We anticipate that sequence-based comparisons and future experimental interrogation of intact proviruses, such as Xq21.33 and others that have yet to be discovered, will give insight into the functionality of similar HML-2 members.

Expression of HML-2 elements has been studied foremost in the context of disease, particularly from tumor-derived tissues (reviewed in 3, 16, 17), but also in otherwise normal human tissues (19). This expression has been shown to exhibit tissue specificity in the form of proviral RNAs from both type 1 and 2 proviruses that have no apparent match to annotated loci (16, 17), implying the presence of transcriptionally active but not yet characterized loci. Although the consequence of such expression is not fully understood, the correlation of HML-2 expression with certain disease states suggests that such variable expression may provide a useful biomarker. Because RNAs corresponding to all previously known polymorphic HML-2 proviruses (and the majority of human-specific elements) have been identified in such assays, additional polymorphic HML-2 copies are likely to be transcribed under certain conditions, justifying the continued characterization of “new” loci and the regulation of their expression. Our analysis of four additional nonreference HML-2 proviruses indicates the presence of discriminatory nucleotide positions within each of these elements (Fig. S6); such sites should aid their assignment in future experimental assays.

Several HML-2 insertions were nearby or within genic regions (Table 1). For example, the 8q24.3c provirus is situated within *COMMD5*, a gene involved in hypertension-related renal repair whose expression is elevated in the kidneys of hypertension-resistant rat models (55). The 4p16d LTR, in ~1.5% of all samples (with highest prevalence in African ancestry), was within the *SLCA29 (GLUT9)* gene; the *GLUT9* uric transporter is a direct target of p53 and is implicated in antioxidant functions (56). Also, the 5q14.1 LTR lies within the *R4SGFR* gene associated with regulation of dopamine neuron activity and reward sensitivity in alcohol use (57). Previous mining of cohort-based WGS data has inferred the 5q14.1 LTR in ~14 to ~30% of those samples (12, 41), although it was detected in ~2.7% of all samples here, possibly explained by the global survey in the data used. Genotyping of this site did permit population distribution estimates, in which we found the highest prevalence in European and American ancestry (up to ~7% of samples), with apparent absence in East Asian individuals (Fig. 2 and Dataset S3). The

relationship between a particular LTR and a biological effect requires further investigations, but these observations serve as a reminder that such insertions may be associated with phenotypic effects in some individuals.

Low levels of replication have been suggested based on the presence of unfixed HML-2 loci (13). Coalescent analysis of globally sequenced LTRs from the K106 provirus (the reference insertion at 3q13.2) has produced an age estimation of 0.15 My based on sequence conservation of that site across sampled individuals (11). Other studies suggest replication until at least 0.25 Mya (12) based on modeling estimations of an expected number of loci, given the number of observed unfixed sites and the proportion of individuals predicted to carry those insertions (12). Although we cannot rule out the possibility of ongoing replication, our comparison of 2-LTR sites suggests a most recent time of insertion at least ~0.67 Mya for those proviruses and we find no evidence of insertions with evidence of more recent formation, noting limitations in properly estimating integration times for recombinant solo-LTRs. The number of rare insertions in our data (15 insertions in <5% and six in <1% of all samples), including the 1p31.1c LTR detected in a single individual and the 2-LTR provirus at 8q24.3c in just a few samples (from the HGDP San and 1KGP Yoruba populations), suggests that additional remaining HML-2 loci are likely to be very rare, specific to groups not yet surveyed, or within low coverage regions of the genome. The rarity of such proviruses, however, is likely to reflect more recently integrated proviruses as well as less time for selective removal of deleterious, pathogenic ones. Continued efforts to analyze additional genome sequences, particularly from previously unstudied populations (particularly of African ancestry), will contribute to the identification of intact and potentially replication-competent proviruses, as is supported by this study.

Our approach shares limitations common to all read-based discovery methods. Given the variability in per-sample coverage, we have likely missed other sites that may be present in one or a few samples or insertions located in otherwise inaccessible regions of the genome. Likewise, other read-based analyses, such as genotyping and derived frequency estimates of each site, must be interpreted with caution, given requirements for read support over each site. Continued improvements in sequencing technologies (longer read lengths) and costs will ameliorate such issues in the future. Such changes will also increase the feasibility of assembly-based approaches, permitting the direct reconstruction of full insertion, ultimately contributing to a more complete picture of all types of genomic variation.

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at this time.

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assistance, however, please let me know if you
have any additional questions.



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